Eur Respir J
1988, 1, 832–838

Oral N-acetylcysteine reduces selected humoral markers of inflammatory cell activity in BAL fluid from healthy smokers: correlation to effects on cellular variables.

A. Eklund*, Ö. Eriksson**, L. Håkansson***, K. Larsson****, K. Ohlsson†, P. Venge***, H. Bergstrand†, A. Björnson††, R. Brattsand††, C. Glennow†††, M. Linden†††, E. Wieslander††

Oral N-acetylcysteine reduces selected humoral markers of inflammatory cell activity in BAL fluid from healthy smokers: correlation to effects on cellular variables. A. Eklund, Ö. Eriksson, L. Håkansson K. Ohlsson, P. Venge, H. Bergstrand, A. Björnson, R. Brattsand, C. Glennow, M. Linden, E. Wieslander.

ABSTRACT: Bronchoalveolar lavage (BAL) was performed on eleven healthy smokers before and after eight weeks of oral treatment with N-acetylcysteine (NAC) 200 mg *t.i.d.* The concentrations of selected eosinophil and neutrophil granule constituents and of selected proteases and protease inhibitors, albumin and endotoxin were determined in the recovered BAL fluid and in plasma or serum samples. In addition, *in vitro* chemotactic activities for neutrophils and eosinophils were assessed in the BAL fluid. Significant reductions in BAL fluid content of lactoferrin (LF), eosinophil cationic protein (ECP), antichymotrypsin (ACT) and chemotactic activity for neutrophils were recorded after NAC treatment. The levels of other examined markers tended to be reduced or were not affected. In serum/plasma, the concentrations of myeloperoxidase (MPO) and elastase were reduced after NAC treatment whereas concentrations of other constituents examined were unaltered. These data, together with previously reported findings, suggest that oral NAC may influence the activity of "inflammatory" cells in the bronchoalveolar space of smokers.

Eur Respir J., 1988, 1, 832–838.

Correspondence: H. Bergstrand, Research & Development Dept, Pharmacological Laboratory, AB Draco, Box 34, S-221 00 Lund, Sweden.

Keywords: Albumin; alveolar macrophage; bronchoalveolar lavage; chemotactic factors; elastase; endotoxin; eosinophil cationic protein; lactoferrin; leukotriene B_4; myeloperoxidase; N-acetylcysteine; phagocytosis; protease inhibitors; smoking; superoxide radical.

Received: November 5, 1987; accepted after revision April 27, 1988.

Oral treatment of patients with chronic bronchitis with N-acetylcysteine (NAC) may lead to a reduction in the rate of exacerbations [1–3]. Although NAC is often referred to as a mucolytic agent [1, 2] or a radical scavenger, the reason for the possible beneficial effect of NAC in chronic bronchitis is unclear.

Chronic bronchitis is often associated with smoking and smoking in turn is associated with an increased incidence of infectious diseases in the lung [4, 5]. Smoking also influences the abundance and activity of "inflammatory" cells in the airways [6]. Thus, the number of cells that can be recovered by lavage from the bronchoalveolar space is markedly higher in smokers than in nonsmokers [7–11]. Moreover, the capacity of alveolar macrophages and peripheral leukocytes to generate oxygen radicals and leukotriene B_4 (LTB_4) when stimulated may be altered for cells from smokers [7, 9, 11–14]. Smoking is also reported to alter proportions of immunoregulatory T-cell subsets in peripheral blood and in bronchoalveolar lavage fluid [11, 15–17]. Therefore in addition to the postulated mucolytic effect of NAC or its supposed interference with mucus hypersecretion [1, 18], the NAC-induced reduction in exacerbation rates in patients with chronic obstructive lung disease could possibly also result from an improvement in the host response to infectious agents. To examine this possibility, we decided to assess whether NAC can directly or indirectly influence inflammatory cells of smokers, particularly cells in the airways. We therefore analysed bronchoalveolar lavage (BAL) fluid and plasma or serum from healthy smokers for selected markers of inflammatory cell activity before and after oral treatment with NAC.

The present report summarizes the study and gives the results recorded for soluble constituents of the BAL fluid. The results recorded with respect to cellular content/composition and functions are reported separately [10, 13]. The results as a whole suggest that the oral NAC treatment may to some extent normalize the alterations in inflammatory cell activity induced by smoking.

* The Dept of Thoracic Medicine, Karolinska Hospital, Solna, Sweden.
** Dept of Drugs, National Board of Welfare, Uppsala, Sweden.
*** Dept of Clinical Chemistry, University Hospital, Uppsala, Sweden.
**** National Institute of Occupational Health, Solna, Sweden.
† The Department of Surgical Patheophysiology, University of Lund, Malucö General Hospital, Malucö.
†† The Pharmacological Department, Research and Develpment, Research and Development Laboratories, AB Draco, Lung Sweden.
††† The Department of Clinical Research, AB Draco, Lung Sweden.

Patients and methods

Subjects

Eleven healthy smokers (four men), with a mean age of 29±2 yrs, participated in the study. All subjects gave their informed consent and the study had the approval of the local Ethics Committee. All had consumed twenty or more cigarettes per day for at least five years and the mean cigarette consumption was 14.5±2.8 pack-years. No subject changed smoking habits markedly during the study. Seven had no respiratory symptoms, the remaining four having productive cough in the mornings. Lung function tests, chest X-ray and ECG were examined [9, 12] before each BAL and did not deviate from normal.

Table 1. – Bronchoalveolar lavage (BAL) fluid and serum/plasma levels of eosinophil cationic protein (ECP), lysozyme, lactoferrin (LF), myeloperoxidase (MPO), anti-leukoprotease (ALP), elatase, albumin, α_1-protease inhibitor (α_1-PI), α_2-macroglobulin (α_2-M), anti-chymotrypsin (ACT), endotoxin and eosinophil and neutrophil chemotactic activity in smoking individuals before and after oral treatment with NAC for eight weeks.

	BAL fluid				Serum/plasma			
	Before NAC n=11	After NAC n=11	Before NAC n=10	After NAC n=10	Before NAC n=11	After NAC n=11	Before NAC n=10	After NAC n=10
ECP	22.6	13.7*	20.8	14.0*	32.7	36.0	30.8	35.9
$\mu g \cdot l^{-1}$	3.2	1.0	2.6	1.0	6.2	5.6	5.8	5.6
Lysozyme	413.0	287.0	322.2	280.5	2802	2397	2743	2387
$\mu g \cdot l^{-1}$	93.8	33.9	19.2	33.3	212	171	204	171
LF	104.5	45.4*	53.8	43.8*	1088	928	956	954
$\mu g \cdot l^{-1}$	50.9	4.4	3.6	4.1	170	97	106	93
MPO	14.5	4.3	6.4	4.7	380.0	244*	342	240
$\mu g \cdot l^{-1}$	8.2	1.1	1.5	1.1	59.6	41	46	41
ALP	36.3	32.6	30.0	33.6	58.7	56.9	57.2	55.5
$\mu g \cdot l^{-1}$	6.8	7.4	2.4	7.3	3.3	3.5	2.9	3.2
Elastase	1.34	0.82	0.96	0.86	68.0	36.1(*)	71.9	37.2*
$\mu g \cdot l^{-1}$	0.41	0.12	0.13	0.11	15.7	5.9	15.2	5.8
Albumin	69	28.7	31	25.8	42.8	46.3**	43.1	46.4*
	38	4.2	2	2.9	0.8	1.4	0.7	1.4
α_1-PI	0.73	0.23	0.19	0.34	1.55	1.64	1.58	1.48
	0.49	0.04	0.04	0.13	0.08	0.07	0.07	0.16
α_2-M	0	0	0	0	73.2	72.7	71.5	70.0
					4.7	6.0	4.4	6.3
ACT	0.085	0.044*	0.052	0.068*	0.355	0.368	0.360	0.356
	0.032	0.008	0.006	0.030	0.032	0.022	0.032	0.022
Endotoxin	167	19	150	21	ND	ND		
$pg \cdot ml^{-1}$	101	11	110	11				
Eosinophil chemotactic activity $\mu m \cdot h^{-1}$	13	6	11	6				
	3	5	2	5	ND	ND		
Neutrophil chemotactic activity $\mu m \cdot h^{-1}$	25	18*	20	17				
	4	4	2	9	ND	ND		

ECP, lysozyme, LF and MPO were examined in serum whereas ALP, elastase, albumin, α_1-PI, α_2-M and ACT were assessed in plasma. Figures given are mean±SEM and are shown for n=11 and n=10 (one individual excluded; see text). The figures given for albumin, α_1-PI and ACT are in $mg \cdot l^{-1}$ (BAL fluid) and in $g \cdot l^{-1}$ (plasma). ND: not determined. *: $p<0.05$; **: $p<0.01$; (*): $p=0.059$.

Table 2. – A schematic summary of effects of oral NAC treatment on selected markers of inflammatory cell activity and proteases/antiproteases in airways (assessed by BAL) and in plasma/serum of healthy smokers

Effect of NAC treatment				
Cellular variables	BAL	Humoral variables	BAL	Plasma/ serum
Total cell yield	o			
Macrophage yield	o	ECP	↓	o
Neutrophil yield	o	Lysozyme	(↓)	o
Eosinophil yield	o	Lactoferrin	↓	o
Lymphocyte yield	↑	Myeloperoxide	(↓)?	↓?
		Anti-leukoprotease	o	o
LTB_4 generation	↑	Elastase	o	↓
Phagocytosis	(↑)	Albumin	o	↑
Superoxide radical generation		α_1-protease inhibitor	o	o
		α_2-macroglobulin	o	o
Spontaneous	o	Anti-chymotrypsin	↓	o
PMA	(↓)	Endotoxin	(↓)	ND
STZ	o	Eosinophil chemotactic activity	(↓)	ND
A23187	↓			
FMLP	↓	Neutrophil chemotactic activity	↓ ?	ND

The data forming the basis for this summary are detailed in refs [9] and [12] and in table 1 of the present report. ↑: denotes significant increase after NAC treatment; ↓: denotes significant decrease after NAC treatment; symbols in brackets denote suggestive but non-significant changes; ?: indicates that this change is not significant if one specific individual is excluded; o: denotes no change

After the first BAL, the subjects received oral medication with NAC (Draco, Lund, Sweden) 200 mg *t.i.d.* for eight weeks. One and a half hours after the last dose the second BAL was performed. The study was performed during October, November, and December.

Bronchoalveolar lavage

Bronchoscopy was performed through the mouth with a flexible fibreoptic bronchoscope (Olympus Type 4B2), under local anaesthesia with 2% lignocaine (Xylocaine®, Astra, Södertälje, Sweden), after premedication with morphine-scopolamine and phenemal. The bronchoscope was wedged in a middle lobe bronchus and 250 ml of sterile saline solution at 37°C was instilled in five aliquots of 50 ml each. After each instillation the fluid was gently aspirated and collected in a siliconized plastic tube. It was strained through a double layer of Dacron net and the volume was measured. The cells were pelleted at 400 g for 5 min at +4°C and then resuspended in RPMI 1640 supplemented with gentamycin (50 $\mu g \cdot ml^{-1}$) and 5% foetal calf serum. After transport (at approximately 4°C), differential and total cell counts, viability tests (Trypan blue) and examination of superoxide and LTB_4 generation and phagocytic capacity were all performed, as has been reported in detail previously [10, 13]. The cell-free BAL fluid supernatant (and plasma/serum samples harvested at the same time for each BAL) were analysed, according to procedures previously described in detail, for eosinophil cationic protein (ECP) [19], lysozyme [20], lactoferrin (LF) [20, 21], myeloperoxidase (MPO) [20, 21], anti-leukoprotease (ALP) [22] and elastase [23]. Albumin, alpha-1-protease inhibitor (α_1-PI), α_2-macroglobulin (α_2-M), and anti-chymotrypsin (ACT) were quantified using electroimmunoassay [24]. Endotoxin was determined in BAL fluid supernatants with the aid of the Limulus assay [25]. Eosinophil and neutrophil chemotactic activities in crude BAL fluid supernatants were determined as described previously [26].

Statistical analyses

Statistical analyses were performed using the RS/1 programme (BBN Software Products Corporation, Cambridge, MA, USA) which utilizes either Student's paired t-test or, if the sample population shows indication of not being normally distributed, Wilcoxon's test on paired observations. Pearson's correlation coefficients were tested against zero with Student's t-test.

Results

BAL fluid and plasma/serum levels of selected granule constituents of inflammatory cells, as well as levels of selected proteases/antiproteases, were examined before and after eight weeks of treatment with oral NAC. Chemotactic activity for neutrophils and eosinophils *in vitro* were assessed in BAL fluid only. The results are shown in table 1. Significant reductions in BAL fluid levels of eosinophil cationic protein (ECP),

Table 3. – Correlation coefficients (r) recorded at examination of correlation between degrees of NAC treatment-induced changes in various examined variables

Variable	Variable	n=11		n=10	
		r	t	r	t
ECP, BAL	MPO, BAL	0.835	4.57**	0.723	2.95*
Lysozyme, BAL	LF, BAL	0.798	3.97**	0.757	3.27*
Lysozyme, BAL	ALP, BAL	0.897	6.10***	0.846	4.49**
Lysozyme, BAL	Endotoxin, BAL	0.741	3.31**	0.649	2.41*
LF, BAL	MPO, BAL	0.849	4.81**	0.496	1.61
LF, BAL	ALP, BAL	0.799	3.98**	0.867	4.92**
LF, BAL	Elastase, BAL	0.766	3.57**	0.040	0.11
LF, serum	MPO, serum	0.862	5.10***	0.900	5.84**
MPO, BAL	Elastase, BAL	0.823	4.34**	0.489	1.59
MPO, serum	Cellular, LTB_4	0.769	3.36**	0.742	2.92*
ACT, BAL	Chemotactic activity, EOS	0.767	3.38**	0.684	2.48*
α_1-PI, plasma	Albumin, BAL	0.778	3.71**		
α_1-PI, BAL	ACT, BAL	0.836	4.30**	0.768	3.17*
α_1-PI, BAL	α_1-PI, plasma	0.913	6.34***	0.865	4.56**

Log (preNAC/postNAC) was calculated for each variable and patient (n=11); for chemotactic activities preNAC-postNAC values were used. Out of 276 examined correlations only those showing significance at the $p<0.01$ level are shown. The corresponding figures obtained when one individual is excluded are given on the right. For variable abbreviations see table 1. *: $0.01<p<0.05$; **: $0.001<p<0.01$; ***: $p<0.001$

lactoferrin (LF), anti-chymotrypsin (ACT) and neutrophil chemotactic activity were recorded, whereas the levels of other variables examined were not significantly affected by the NAC treatment. In plasma/serum, only the levels of elastase, myeloperoxidase and albumin were significantly affected by the treatment. The concentrations in BAL fluid or serum/plasma of ECP, LF, ACT, MPO, and elastase recorded after NAC treatment approached the values normally recorded for these constituents in samples from apparently healthy nonsmokers (data not shown).

Two of the individuals showed signs of upper airway infection at the first bronchoscopy examination. For one of these individuals, high levels were recorded for all BAL fluid humoral markers (but not for cellular activity markers [10, 13]). If the patient with these high levels is excluded from the results and calculations are performed based on the remaining ten individuals, significant reductions of BAL fluid ECP, LF and ACT and of plasma levels of elastase are still recorded, whereas the significant reductions in BAL fluid level of neutrophil chemotactic activity and serum level of MPO induced by NAC treatment are not observed (table 1).

The results recorded in the present study with respect to cell recovery, differential cell count and viability, with respect to oxygen radical generation, LTB_4 generation and phagocytosis have all been detailed elsewhere [10, 13]. In table 2 we show a schematic summary of the findings of the present study.

The conspicuous finding of this study seems to be that oral treatment of smokers with NAC tends to reduce the levels of supposed markers of inflammatory cell activities in BAL fluid and to normalize smoking-induced activation/deactivation of selected BAL cell function(s). We therefore examined (with the recorded data as the basis) whether a correlation existed between the various effects of NAC. For that purpose log (preNAC/postNAC) was calculated for each variable and patient as a measure of the change induced by NAC treatment; for the chemotactic activities preNAC-postNAC values were used since for these variables negative recordings could occur. We then examined the linear correlation between (preNAC/postNAC) values for th e various variables. In these calculations, figures for cellular yield and for α_2-macroglobulin were not included; as mentioned previously the latter is not detectable in BAL fluid. Furthermore, superoxide radical generation was represented by figures recorded at triggering with PMA. Since 24 different humoral and cellular variables were thus followed, a total of 276 different correlations were examined. Fourteen of these (collected in table 3) were found to be significant at the levels of $p<0.01$. Two to three of the fourteen correlations would thus be expected to have occurred by chance. However, it is of interest that the changes, apparently induced by NAC treatment, in BAL fluid levels of humoral markers of granulocyte activity often correlated significantly, as did changes induced by NAC treatment in the serum levels of lactoferrin and myeloperoxidase. Conversely, no convincing correlation was recorded between effects of NAC treatment on any pair of examined variables of cellular activation (LTB_4, superoxide generation or phagocytic activity) or between such an activity and endotoxin levels in BAL [10, 13].

Discussion

A beneficial effect of oral treatment with N-acetylcysteine on the exacerbation rate in patients with chronic bronchitis has been documented in at least two multicentre studies [1, 2]. A third study [3], which examined patients with more advanced disease reported similar results but the difference did not reach statistical significance.

The reason for such an effect of NAC on the exacerbation rate in patients with chronic bronchitis is not known. However, it is now realized that following oral treatment, NAC can hardly be considered to exert mucolytic activity in the airways [27]. Since the possibility that smoking may induce changes in the immune and inflammatory system(s) and, therefore, may contribute to the disease is now supported by numerous studies (for reviews see [6, 11]) we speculated that NAC could in some way reverse a smoke-induced defect in an "antimicrobial" host response. To examine this possibility we assessed the influence of oral NAC treatment on selected variables of the airway immune response (in a broad sense) in healthy smokers. The present report gives the results with respect to the effects of NAC treatments on examined humoral components. Previous reports [10, 13] have detailed effects of the treatment recorded for cellular constituents of BAL fluid. We found that NAC treatment significantly reduced the BAL fluid levels of the eosinophil and the neutrophil granule constituents ECP and lactoferrin and also seemed to reduce those of some (but not all) of the other granulocyte constituents examined. Serum/plasma levels of examined variables were not significantly affected, with the exception of myeloperoxidase and, possibly, elastase (both neutrophil constituents) and albumin.

Another conspicuous finding in this study was that NAC treatment of smokers did not affect BAL or plasma/serum concentrations of proteases/protease inhibitors examined, with two possible exceptions. These were elastase (previously reported to be elevated in BAL fluid as well as in plasma/serum of smokers [28, 29]) and anti-chymotrypsin. The BAL fluid concentration of both of these components tended to be reduced following NAC treatment (table 1). On the other hand, immunoreactive α_1-protease inhibitor level in BAL was not changed by NAC treatment; in fact, the level of this component (and possibly also that of ALP) was, if anything, higher in BAL fluid of smokers than in that of healthy nonsmokers (unpublished data). This is in apparent contrast to previous reports of reduced activity of α_1-protease inhibitor in BAL from smokers compared to normals [30–32].

Together with the results recorded on cellular variables [10–13] (table 2) and those independently recorded for nonsmoking individuals (unpublished results), the general finding of this study is that NAC treatment tends to normalize a dysfunction induced by cigarette smoke. Such an interpretation is underlined by the recordings of significant correlations between effects of NAC treatment on different but apparently related variables, *e.g.* LF serum and MPO serum, whereas apparently "false" correlations (those which are difficult to explain) are few (table 3). In this context we are aware of the fact that lactoferrin in BAL is not a neutrophil-specific constituent but is also produced by serous cells.

The correlation analysis also provides other important information. Firstly, since no correlation exists between any examined cellular activity and endotoxin levels in BAL, the altered cellular activity previously recorded for cells from smokers [10, 13] is less likely to be due to endotoxin "contamination" of the BAL fluid. Secondly, several "expected" or easily explained correlations between humoral, supposedly granule-derived variables are noted but few, if any, between any humoral and cellular variables or between different variables reflecting cellular activity. Therefore, the examined cellular activities may be similarly triggered and/or regulated by independent mechanisms. It has previously been observed in studies *in vitro* that the system for neutrophil superoxide generation is physically separate and induced independently from that of degranulation [33, 34]; in the human basophil histamine release system, various secretagogues induce mediator release independently [35, 36]. The present data therefore seem to indicate that various functions of a granulocyte/mononuclear cell may be regulated independently *in vivo* also.

The basis for the suggested effect of NAC remains unclear. However, the finding that distinct examined variables, like BAL content of the eosinophil marker ECP and the supposed neutrophil constituent lactoferrin are affected by the NAC treatment suggests that either NAC exerts an indirect effect on granulocyte function, *e.g.* by influencing production of principles governing the activity of granulocytes, or that NAC may influence a biochemical pathway which is important in the function of eosinophils as well as neutrophils. The former alternative is substantiated by findings that components of the smoke may directly induce activation of cells or may stimulate lymphocytes to produce factors which in turn activate phagocytes [11]. With respect to, *e.g.* oxygen radical production, different lymphocyte-derived principles have been attributed roles as inducers of such a primed state. γ -interferon is a necessary and sufficient inducer of that activity in human macrophages [37]. Conversely, ß-interferon is reported to reduce macrophage oxygen radical generating capacity [38]. Therefore, one speculative explanation for the effect of NAC treatment would be that NAC affects/normalizes the production by mononuclear cells of factors with chemotactic and/or priming activity for effector cells in the airways. It is also possible that NAC treatment influences the activity of granulocytes more directly leading to reduced levels of their products. Such a possibility is strengthened by, *e.g.*, the recorded reduction by NAC treatment of elastase and myeloperoxidase serum/plasma levels (table 1). This effect, in turn, is significantly correlated to that on serum lactoferrin (table 3). Since neither NAC nor an increased cysteine level can clearly be demonstrated in BAL fluid after oral treatment with the drug [26], any action of NAC or a NAC derivative on peripheral granulocytes would seem more probable than an action locally in the lung.

At the biochemical level also, the explanations for the effect(s) seen with NAC are not known. NAC has previously been reported to modulate various biochemical parameters of rat alveolar macrophages [39]. Recent experience also indicates that NAC markedly enhances the effectiveness of nitro compounds with respect to vascular smooth muscle relaxation [40, 41] and inhibition of platelet aggregation [42]. These effects of nitro compounds are thought to be partly due to their capacity

to increase generation of cyclic guanosine monophosphate (cGMP) [43]. Since NAC treatment is claimed to lead to increased plasma levels of cysteine [44] one may speculate that NAC in some way fulfills the requirement for SH-compounds which seems to exist at activation of soluble cellular guanylate cyclase [43].

Only further detailed studies on the influence of NAC on the activity of immune cells in smokers and patients with chronic bronchitis, together with biochemical examinations of its supposed influence on guanylate cyclase can clarify whether this may be the basis for the observed effect of the drug.

Acknowledgements : The skilful technical assistance of R. Hernbrand, K. Lindblad and M. Rosengren is gratefully acknowledged. Our sincere thanks to Dr T. Persson who gave expert statistical advice. Mrs U. Larsson kindly typed the manuscript. Grants from the Swedish National Association against Heart and Chest Diseases and the Swedish Medical Research Council are gratefully acknowledged. AB Draco is a subsidiary to AB Astra, Södertälje, Sweden.

References

1. Multicentre Study Group. – Long-term oral acetylcysteine in chronic bronchitis. A double-blind controlled study. *Eur J Respir Dis*, 1980, 61 (Suppl. 111), 93–108.
2. Boman G, Bäcker U, Larsson S, Melander B, Wåhlander L. – Oral acetylcysteine reduces exacerbation rate in chronic bronchitis. Report of a trial organized by the Swedish Society for Pulmonary Disease. 1983, 64, 405–415.
3. British Thoracic Society Research Committee. – Oral N-acetylcysteine and exacerbation rates in patients with chronic bronchitis and severe airways obstruction. *Thorax*, 1985, 40, 832–835.
4. Haynes WF Jr, Krstlovic VJ, Bell ALL Jr. – Smoking habit and incidence of respiratory tract infections in a group of adolescent males. *Am Rev Respir Dis*, 1966, 93, 730–735.
5. Darnell JL, Anderson DO, Kinnis C. – Cigarette smoking and respiratory infections in a class of student nurses. *New Engl J Med*, 1966, 274, 979–984.
6. Janoff A, Pryor WA, Bengali ZH. – NHLBI Workshop Summary. Effects of tobacco smoke components on cellular and biochemical processes in the lung. *Am Rev Respir Dis*, 1987, 136, 1058–1064.
7. Finch GL, Fisher GL, Hayes TL, Golde DW. – Surface morphology and function at studies of human alveolar macrophages from cigarette smokers and non-smokers. *Res J Reticulo Soc*, 1982, 32, 1–23.
8. Ando MY, Sugimoto M, Nishi R, Suga M, Hovio S, Kokvogi H, Shimazu K, Araki S. – Surface morphology and function of human pulmonary alveolar macrophages from smokers and non-smokers. *Thorax*, 1984, 39, 850–856.
9. Hoidal JR, Fox RB, LeMarbe PA, Perri R, Repine JE. – Altered oxidative metabolic responses *in vitro* of alveolar macrophages from asymptomatic cigarette smokers. *Am Rev Respir Dis*, 1981, 123, 85–89.
10. Linden M, Wieslander E, Eklund A, Larsson K, Brattsand R. – Effects of oral N-acetylcysteine on cell content and macrophage function in bronchoalveolar lavage from healthy smokers. *Eur Respir J*, (in press).
11. Holt PG. – Review article. Immune and inflammatory function in cigarette smokers. *Thorax*, 1987, 42, 241–249.
12. Ludwig PW, Hoidal JR. – Alterations in leukocyte oxidative metabolism in cigarette smokers. *Am Rev Respir Dis*, 1982, 126, 977–980.
13. Bergstrand H, Björnson A, Eklund A, Hernbrand R, Larsson K, Linden M, Nilsson A. – Stimuli-induced superoxide radical generation *in vitro* by human alveolar macrophages from smokers: modulation by N-acetylcysteine treatment *in vivo*. *J Free Rad Biol Med*, 1986, 2, 119–127.
14. Wieslander E, Linden M, Håkansson L, Eklund A, Blaschke E, Brattsand R, Venge P. – Human alveolar macrophages from smokers have an impaired capacity to secrete LTB_4 but not other chemotactic factors. *Eur J Respir Dis*, 1987, 71, 263–272.
15. Costabel U, Bross KJ, Reuter C, Ruhle K-H, Matthys H. – Alterations in immunoregulatory T-cell subsets in cigarette smokers. A phenotypic analysis of bronchoalveolar and blood lymphocytes. *Chest*, 1986, 90, 39–44.
16. Smart YC, Cox J, Roberts TK, Brinsmead MW, Burton RC. – Differential effect of cigarette smoking on recirculating T lymphocyte subsets in pregnant women. *J Immunol*, 1986, 137, 1–3.
17. Weissman DN, de Shazo RD, Banks DE. – Modulation of natural killer cell function by human alveolar macrophages. *J Allergy Clin Immunol*, 1986, 78, 571–577.
18. Rogers DF, Jeffery PK. – Inhibition of oral N-acetylcysteine of cigarette smoke-induced "bronchitis" in the rat. *Exp Lung Res*, 1986, 10, 267–283.
19. Venge P, Roxin L-E, Olsson I. – Radioimmunoassay of human eosinophil cationic protein. *Br J Haematol*, 1977, 37, 331–335.
20. Venge P, Hällgren R, Stålenheim G, Olsson I. – Effect of serum and cations on the selective release of granular proteins from human neutrophils during phagocytosis. *Scand J Haematol*, 1979, 22, 317–326.
21. Olofsson T, Olsson I, Venge P, Elgegors B. – Serum myeloperoxidase and lactoferrin in neutropenia. *Scand J Haematol*, 1977, 18, 73–80.
22. Fryksmark U, Ohlsson K, Rosengren M, Tegnér H. – A radioimmunoassay for measurement and characterization of human antileukoprotease in serum. *Hoppe-Seyler's Z Physiol Chem*, 1981, 362, 1273–1277.
23. Ohlsson K, Olsson A-S. – Immunoreactive granulocyte elastase in human serum. *Hoppe-Seyler's Z Physiol Chem*, 1978, 359, 1531–1539.
24. Laurell C-B. – Electroimmunoassay. *Scand J Clin Lab Invest*, 1972, 29 (Suppl.), 21–37.
25. Friberger R. – The design of a reliable endotoxin test. *In:* Bacterial Endotoxins: structure, biomedical significance and detection with the Limulus amebocyte test. Alan. R. Liss. Inc., New York, 1985, pp. 139–149.
26. Håkansson L, Westerlund D, Venge P. – A new method for the measurement of eosinophil migration. *J Leukocyte Biol*, 1987, 42, 689–696.
27. Cotgreave IA, Eklund A, Larsson K, Moldéus PW. – No penetration of orally administered N-acetylcysteine into bronchoalveolar lavage fluid. *Eur J Respir Dis*, 1987, 70, 73–77.
28. Janoff A, Raju L, Dearing R. – Levels of elastase activity in bronchoalveolar fluids of healthy smokers and non-smokers. *Am Rev Respir Dis*, 1983, 127, 540–544.
29. Harris JO, Olsen GN, Castle JR, Maloney AS. – Comparison of proteolytic enzyme activity in pulmonary alveolar macrophages and blood leukocytes in smokers and non-smokers. *Am Rev Respir Dis*, 1975, 111, 579–586.
30. Gadele J, Felb GA, Crystal RG. – Cigarette smoking induced functional antiprotease deficiency in the lower respiratory tract of humans. *Science*, 1979, 206, 1315–1316.
31. Carp H, Janoff A. – Inactivation of bronchial mucous prokinase inhibitor by cigarette smoke and phagocyte-derived oxidants. *Exp Lung Res*, 1980, 1, 225–237.

32. Carp H, Miller F, Hoidal JR, Janoff A. – Potential mechanism of emphysema: alpha-1-prokinase inhibitor recovered from lungs of cigarette smokers contains oxidized methionine and has decreased elastase activity. *Proc Nat Acad Sci USA*, 1982, 79, 2041–2045.
33. Cox CC, Dougherty RW, Ganong BR, Bell RM, Niedel JE, Snyderman R. – Differential stimulation of the respiratory burst and lysosomal enzyme secretion in human polymorphonuclear leukocytes by synthetic diacylglycerols. *J Immunol*, 1986, 136, 4611–4616.
34. Lutter R, van Zwieten R, Weening RS, Hamers MC, Roos D. – Cytochrome B, flavins, and ubiquinone-50 in enucleated human neutrophils (polymorphonuclear leukocyte cytoplasts). *J Biol Chem*, 1984, 259, 9603–9606.
35. Siraganian RP, Hook WA. – Mechanisms of histamine release by formyl methionine containing peptides. *J Immunol*, 1977, 119, 2078–2083.
36. Findlay SR, Lichtenstein LM. – Basophil "release-ability" in patients with asthma. *Am Rev Respir Dis*, 1980, 122, 53–59.
37. Nathan CF, Murray HW, Wiebe M, Rubin BY. – Identification of interferon as the lymphokine that activates human macrophage oxidative metabolism and automicrobial activity. *J Exp Med*, 1983, 158, 670–689.
38. Boraschi D, Ghezzi P, Pasqualetto E, Salmona M, Nencioni L, Soldateschi D, Villa L, Tagliabue A. – Interferon decreases production of hydrogen peroxide by macrophages: correlation with reduction of suppressive capacity and of antimicrobial activity. *Immunology*, 1983, 52, 359–368.
39. De Flora S, Romano M, Basso C, Bagnasco M, Cesarone CF, Rossi GA, Morelli A. – Detoxifying activities in alveolar macrophages of rats treated with acetylcysteine, diethyl maleate and/or aroclor. *Anticancer Res*, 1986, 6, 1009–1012.
40. Horowitz JD, Autman EM, Lovell BH, Barry WH, Smith TW. – Potentiation of the cardiovascular effects of nitroglycerin by N-acetylcysteine. *Circulation*, 1983, 68, 1247–1253.
41. Winniford MD, Kennedy PL, Wells PJ, Hillis LD. – Potentiation of nitroglycerin-induced coronary dilatation by N-acetylcysteine. *Circulation*, 1986, 73, 138–142.
42. Loscalzo J. – N-acetylcysteine potentiates inhibition of platelet aggregation by nitroglycerin. *J Clin Invest*, 1985, 76, 703–708.
43. Ignarro LJ, Kadowitz PJ. – The pharmacological and physiological role of cyclic GMP in vascular smooth muscle relaxation. *Ann Rev Pharmacol Toxicol*, 1985, 25, 171–191.
44. Aylward M. – Report on a study on the clinical therapeutic evalution of N-acetyl-L-cysteine (Fluimucil) in the treatment of patients with chronic obstructive bronchitis: a balanced double-blind trial with placebo control. Cited as personal communication in ref.[1].

RÉSUMÉ: Le lavage broncho-alvéolaire (BAL) a été pratiqué chez 11 fumeurs bien portants, avant et après un traitement oral de huit semaines au moyen de N-acétylcystéine (NAC; 200 mg trois fois par jour). Les concentrations des constituants sélectionnés des granules éosinophiles et neutrophiles, ainsi que ceux de protéases et d'inhiditeurs de protéases d'albumine et d'endotoxine, ont été déterminées dans le liquide de lavage ainsi que dans le plasma ou le sérum des individus concernés. En outre, les activités chemotactiques pour les neutrophiles et les éosinophiles ont été mesurées dans le liquide de lavage in vitro. Des diminutions significatives du contenu du liquide de lavage broncho-alvéolaire en lactoferrine (LF), en protéines cationiques éosinophiles, en anti-chymotrypsine et en activité chemotactique pour les neutrophiles, ont été décelées après traitement à la NAC. Quant aux autres marqueurs examinés, leurs niveaux soit tendaient à diminuer, soit n'étaient pas modifiés. Dans le sérum/plasma, les concentrations de myéloperoxidase (MPO) et d'élastase, ont été respectivement diminuées après traitment à la NAC, alors que celles des autres constituants examinés restaient inchangées. Ces donnés, associées à des résultats rapportés antérieurement concernant l'influence du traitement à la NAC sur le nombre et la fonction des diverses cellules du lavage broncho-aléolaire, suggèrent que la NAC orale peut influencer l'activité des cellules "inflammatoires" dans l'espace broncho-alvéolaire des individus fumeurs.