

## N-acetylcysteine in cystic fibrosis and *Pseudomonas aeruginosa* infection: clinical score, spirometry and ciliary motility

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*N-acetylcysteine in cystic fibrosis and Pseudomonas aeruginosa infection: clinical score, spirometry and ciliary motility. G. Stafanger, C. Koch.*

**ABSTRACT:** The effect of peroral N-acetylcysteine (NAC) in patients with cystic fibrosis (CF) and chronic pulmonary *Pseudomonas aeruginosa* infection was studied in 52 patients in a double-blind, placebo-controlled, cross-over trial of two, 3 month durations. Active treatment consisted of NAC, 200 mg  $\times$  3 daily (patients weighing  $<30$  kg) or 400 mg  $\times$  2 daily ( $>30$  kg). The effect was evaluated by a subjective clinical score, weight, sputum bacteriology, blood leucocyte count, sedimentation rate, titres of specific antimicrobial antibodies, lung function parameters and measurement of nasal ciliary function *in vitro*. 31 patients completed the study. No significant differences in lung function or subjective clinical scores were seen between NAC and placebo for the study group as a whole. Patients with peak expiratory flow rate (PEFR) below 70% of predicted normal values showed a satisfactory significant increase in PEFR, forced vital capacity (FVC) and forced expiratory volume in one second (FEV<sub>1</sub>) during NAC treatment. No effect of NAC on ciliary activity was observed.

*Eur Respir J.*, 1989, 2, 234-237.

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Keywords: ciliary motility, cystic fibrosis, N-acetylcysteine.

Accepted: October, 12, 1988.

Orally administered N-acetylcysteine (NAC) has by some authors been demonstrated to have a positive clinical effect in patients with chronic bronchitis [1-3] when looking at changes in sputum consistency, ease of expectoration, and decrease in number of acute exacerbations. Other studies, however, have failed to demonstrate any effect of NAC in such patients [4].

Mucolytics, either as inhalants or taken orally, have for many years been part of the daily routine treatment in patients with cystic fibrosis (CF) to facilitate expectoration. Several studies of the effect of NAC, taken orally by CF patients have been performed, but the results are diverse, ranging from no effect [5, 6] to a slightly positive effect [7].

Recently, we have studied the effect of peroral NAC in patients with CF without chronic pulmonary *Pseudomonas aeruginosa* infection (CPPI) [8]. An improved spirometry reading was seen when the drug was taken in the autumn, *i.e.* the period of the year when the patients suffered most from lower airway infections. Although these patients did not have a very big sputum production and generally were in good health, a statistically significant improvement of the spirometric values was seen during NAC treatment in the most severely ill patients. A similar study was then performed in CF patients with CPPI, having a more advanced disease with considerably more sputum production. As in the previous study the ciliary function *in vitro* was assessed, since a decrease in ciliary beating frequency (CBF) has been demonstrated when ciliated cells were perfused with NAC [9].

### Patients, materials and methods

#### Study design

The study was a double-blind, placebo-controlled, cross-over trial, consisting of 2 periods of 3 months duration, during which the patients were randomly allocated to receive either NAC or placebo. The patients entered the study just after the end of a stay in hospital for treatment against *Pseudomonas aeruginosa* infection. These treatments are given regularly to all CF patients with CPPI approximately every 3rd month, and on admission to hospital for the next treatment they stopped the intake of NAC or placebo, starting on period 2 when again discharged from hospital.

Informed consent was given by all patients and the study has received approval from the local Ethical Committee.

#### Patients

Fifty-two CF patients with CPPI entered the study and 31 completed (17 males, 14 females, mean age 15.8 yr (7-33)). Patients with a past history of peptic ulcer, liver or kidney disease, and pregnant patients were not included in the study.

The diagnosis of CF was based upon a typical clinical history and several quantitative sweat tests. All the patients had CPPI and their clinical condition ranged from rather poor, with a lung function, as judged by



spirometry, below 40% of predicted normal values for age and height, to good with normal lung function.

During the study the patients followed their normal daily routine consisting of lung physiotherapy (mask-PEP=face mask with positive expiratory pressure), supply of pancreatic enzymes and vitamins, and administration of antimicrobials when needed. Inhalation treatment with carbamide was discontinued in all patients prior to entry. Most patients inhaled isotonic NaCl with or without varying amounts of salbutamol, and in individual patients, this was kept constant throughout the study. 13 patients inhaled  $10^6$  IU Colistin  $\times 2$  daily throughout the study [10]. All patients were seen once a month for clinical control and spirometry. Blood and nasal scrapings were sampled at the start and at the end of each period.

All patients were seen at approximately the same time of the day each visit, which should make the lung function parameters comparable, particularly in patients treated with bronchodilators. At each monthly visit a sample of expectoration was analysed for bacteria. If bacterial cultures were positive, oral antibiotics for other than *Pseudomonas aeruginosa* were prescribed routinely for fourteen days.

Twenty of the patients were planned to have their ciliary function tested at the start of the study and at the end of each period. Out of these, 11 completed the tests.

#### Treatment

Active treatment consisted of NAC (ASTRA A/S), 200 mg 3 times daily (patients weighing <30 kg), or 400 mg twice daily (>30 kg). Placebo tablets contained bicarbonate only.

#### Clinical assessments

Once a week the patients completed a chart in which subjective parameters were scored as shown previously [8].

Once a month the treatment was evaluated in terms of the subjective score, weight, sputum bacteriology, and pulmonary function parameters (forced vital capacity (FVC), forced expiratory volume in one second ( $FEV_1$ ), and peak expiratory flow rate (PEFR)), recorded on an electronic spirometer (Spirotron, Dräger).

At the start of the study and at the end of each three month period a blood test was examined for total white blood cell count (WBC), sedimentation rate (ESR), and titres of specific antibodies to *Staph. aureus*, *H. influenza* and *Pseudomonas aeruginosa*.

#### Ciliary function

The nasal ciliary function was studied at the start of the study and at the end of each three month period. Determination of the ciliary function was performed as previously described [11], using an anophthal phase contrast microscope equipped with a microphotometer which transforms the interference of light caused by the ciliary

beating to a curve on a mingograph which depicts the frequency as well as the beating pattern (degree of synchrony between individual cilia).

#### Statistical methods

We used the Wilcoxon test for paired differences and 5% was considered significant.

#### Results

Twenty-one patients were excluded for various reasons as shown in table 1. Ten were excluded because of poor co-operation. Whilst receiving NAC one patient developed Quincke's oedema and one other exanthema. The symptoms disappeared in both cases when the treatment was stopped. Two patients, one while receiving NAC, the other placebo, complained of abdominal pain. One felt that she coughed more frequently and less productively during treatment with NAC and stopped the intake. Two patients were excluded because of major exacerbations and 2 because they had inhalation with colistin put on top of their regular daily treatment during the investigation; all 4 were receiving placebo. The last two who also received placebo just did not like the taste.

Table 1. - No. of patients who dropped out of the study and the various reasons for doing so

	Treatment		Total
	NAC	Placebo	
Quincke's oedema	1		1
Exanthema	1		1
Exacerbation		2	2
Abdominal pain	1	1	2
Change in antibiotic treatment		2	2
Increased non-productive coughing	1		1
Disliked the taste		2	2
Poor co-operation	5	5	10
Total	9	12	21

The results of the subjective clinical scores showed no significant difference between NAC and placebo. For the whole patient material there was a non significant trend towards an improvement of spirometric values after 3 months of NAC treatment compared to placebo treatment. For patients with base-line PEFR less than 70% of predicted values for sex, age and height statistically significant improvement was found both in peak expiratory flow rate (PEFR), forced vital capacity (FVC) and forced expiratory volume in one second ( $FEV_1$ ), after NAC treatment, but not after placebo, as shown in table 2.

When asked about preference only 18 patients answered. Thirteen preferred NAC, 3 placebo and 2 did not know. Two patients preferring NAC, commented on their answer: one had no blood in the mucus in that period, the other found the mucus was looser.

None of the other parameters, including blood tests, revealed any differences between NAC and placebo.



The results of measurements of ciliary activity are shown in table 3. No difference between base-line values and values after treatment with NAC or placebo was seen in any of the parameters measured.

ally better pulmonary condition [8], and NAC was seen to improve the lung function in patients receiving the drug during autumn/winter, but not during summer. The same group of patients also had lower spirometric values

Table 2. – Difference in % between baseline values of lung function parameters and values after treatment with N-acetylcysteine (NAC) and placebo. Patients with baseline PEFR values less than 70% of predicted for sex, age and height

n	PEFR		FVC		FEV <sub>1</sub>	
	NAC-BI %*	Pla-BI %	NAC-BI %	Pla-BI %	NAC-BI %	Pla-BI %
1	18	-2	-2	-7	15	5
2	11	-27	5	-23	8	-25
7	28	-11	9	-8	11	-20
11	17	-9	0	-1	0	-3
20	26	9	-10	-15	2	2
26	6	3	14	9	12	6
33	-17	-2	18	33	-3	-6
35	30	-40	46	-44	16	-48
40	34	13	80	32	59	36
45	-7	-10	11	-16	6	-6
Total	146	-76	171	-70	196	-59
mean	14.6	-7.6	17.1	-7.0	19.6	-5.9
	p<0.05	NS	p<0.05	NS	p<0.02	NS

PEFR: peak expiratory flow rate; FVC: forced vital capacity; FEV<sub>1</sub>: forced expiratory volume in one second; NS: not significant; \*: the difference is given in % of baseline values (BI).

Table 3. – Ciliary beating frequency (CBF), ciliary beating pattern and ciliary motility as base-line values and as values after three months of treatment with N-acetylcysteine (NAC) and placebo

	CBF Hz <sup>1</sup>		Synchronicity grade <sup>2</sup>		Motility % <sup>3</sup>	
	Mean	Range	Mean	Range	Mean	Range
Base-line	6.3	(3.7–10.4)	0.9	(0.6–2.4)	89	(50–100)
NAC	6.3	(4.7–8.2)	0.8	(0.3–1.3)	96	(89–100)
Placebo	7.3	(5.0–8.9)	0.6	(0.2–1.3)	94	(68–100)

<sup>1</sup>Beats per second; <sup>2</sup>Synchronicity grade: PEDERSEN and MYGIND [12]; <sup>3</sup>Cells with motile cilia in % of all ciliated cells counted.

## Discussion

NAC has for many years been used as a mucolytic, either as inhalant or administered perorally. In patients with bronchitis one study showed that NAC eased the expectoration and improved PEFR significantly after intake of 600 mg·day<sup>-1</sup> during a four weeks period [1]. Exacerbation rate in such patients was found to decrease after intake of 400 mg·day<sup>-1</sup> of NAC in one study [2] and after 600 mg·day<sup>-1</sup> every second day in another [3].

In patients with cystic fibrosis STEIL and NIESSEN [7] found a significant improvement in FVC in patients with an initial FVC below 75% of predicted value when given 10–30 mg·kg<sup>-1</sup>·day<sup>-1</sup> for 6 months. Two studies, however, found no effect, one [5] when giving 9.5 mg·kg<sup>-1</sup>·day<sup>-1</sup> of NAC for 14 days, and one [6] 600 mg·day<sup>-1</sup> for three months.

The present study was performed as part of a larger double-blind, cross-over, placebo-controlled investigation on the effect of oral NAC in CF patients. The first part was performed in 41 patients without CPPI, and gener-

ally better pulmonary condition [8], and NAC was seen to improve the lung function in patients receiving the drug during autumn/winter, but not during summer. The same group of patients also had lower spirometric values

and the conclusion of that study was that there might be a positive effect of NAC in patients with more severe illness during the period of the year with most respiratory infections. The present investigation was performed in CF patients with chronic pulmonary *Pseudomonas* infection. They were generally older (mean 15.8 yrs) than the patients in the previous study (mean 9.5 yrs) and they generally had more productive cough. Their ventilatory capacity ranged from rather poor (<40% of predicted value) to normal. The patients entered successively during the whole year, and this made it impossible to investigate for seasonal effect as in the previous study. A significant difference between base-line values and values after treatment with NAC was, however, found for PEFR, FVC and FEV<sub>1</sub> in the patients with a PEFR below or equal to 70%. This result is very much in agreement with STEIL and NIESSEN [7] who found a significant improvement in FVC in patients with a initial FVC below 75% of predicted value.

*In vitro* studies have shown that NAC reduces the



viscosity of mucoprotein solutions by cleaving glycoproteins [13, 14], but to reduce glycoproteins in the sputum NAC has to penetrate into the sputum. That this does happen when administered perorally has been shown by two investigators [15, 16] after administration of 100 mg NAC as a single dose and 200 mg t.i.d. respectively. Recently COTGREAVE *et al.* [17], however, found no penetration of orally administered NAC into bronchoalveolar lavage fluid after 2 weeks of treatment with 600 mg of NAC daily.

Human nasal ciliated epithelium, exposed to NAC in concentrations above 2 mg·ml<sup>-1</sup>, exhibits decreased ciliary beating frequency [9]. We could not, however, demonstrate any such effect on nasal ciliary function *in vitro* following oral intake, neither in this study nor in the previous [8]. This can be explained either by the dose being too low or by NAC not penetrating into the sputum.

As in our previous study [8] this investigation indicates a positive clinical effect of NAC at least in the more severely ill CF patients. How it exerts this effect is still unknown. Further studies on a possible penetration of orally administered NAC into bronchial secretions are warranted. Dampening of inflammation through inhibition of granulocyte derived toxic oxygen radicals may be the mechanism [18, 19] and further *in vitro* and *in vivo* studies on the possible anti-inflammatory effect of NAC in CF patients are warranted.

**Acknowledgements:** Miss Else Mørkassel is warmly thanked for her brilliant technical work. The study was supported by ASTRA A/S, Copenhagen.

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*La N-acétylcystéine dans la fibrose kystique et l'infection par Pseudomonas aeruginosa: scores cliniques, spirométrie et motilité ciliaire. G. Stafanger, C. Koch.*

**RÉSUMÉ:** Les effets de la N-acétylcystéine perorale (NAC), chez des patients atteints de fibrose kystique (CF) et d'infection pulmonaire chronique à *Pseudomonas aeruginosa*, ont été étudiés chez 52 patients au cours d'un essai en double aveugle, avec contrôle par placebo, et permutation croisée, pendant 2 fois 3 mois. Le traitement actif a consisté en 3 × 200 mg de NAC par jour (chez des patients pesant moins de 30 kilos) et 2 × 400 mg par jour (chez ceux pesant plus de 30 kilos). L'efficacité a été évaluée par un score clinique subjectif, le poids, l'examen bactériologique de l'expectoration, la leucocytose, la vitesse de sédimentation, les taux d'anticorps antimicrobiens spécifiques, les paramètres fonctionnels pulmonaires, et la mesure de la fonction ciliaire nasale *in vitro*. L'étude a été complétée chez 31 patients. L'on n'a observé aucune différence significative dans la fonction pulmonaire ou les scores cliniques subjectifs entre les groupes NAC et placebo pour l'ensemble des patients. Les patients dont le débit expiratoire de pointe était à moins de 70% des valeurs théoriques, ont montré une augmentation significative et satisfaisante du débit expiratoire de pointe, de la capacité vitale forcée et du VEMS, au cours du traitement par NAC. L'on n'a observé aucun effet de la NAC sur l'activité ciliaire.

*Eur Respir J*, 1989, 2, 234–237.