Additive effect of dornase alfa and Nacystelyn on transportability and viscoelasticity of cystic fibrosis sputum

Feng Sun MD PhD¹, Shusheng Tai PhD¹, Thomas Lim BSc¹, Ulrich Baumann MD², Malcolm King PhD FCCP¹

OBJECTIVE: To investigate the effect of dornase alfa (DA), Nacystelyn (NAL) and their combination on mucociliary transportability and mucus viscoelasticity of cystic fibrosis (CF) sputum, and to assess whether the combination possesses an additive effect.

DESIGN: Determination of transportability in frog palate and viscoelasticity in vitro.

SETTING: Research laboratory at a medical centre.

PATIENTS: Sputa from 15 patients with CF, chronically infected with Pseudomonas aeruginosa, were studied.

INTERVENTIONS: Sputum samples were incubated without any drug solution as a control, and with normal saline, DA, NAL and a mixture of DA and NAL in concentrations approximating those achieved in clinical practice.

RESULTS: Normal saline (10% volume) by itself had a small effect on CF sputum transportability with a mean increase of 9%, and on viscoelasticity with a mean of decrease of 0.22 log units, respectively, compared with control (incubation without saline). DA (200 nM) further increased the transportability by a mean of 35% versus saline and decreased viscoelasticity by a mean of 0.30 log units. NAL (100 µM) increased the transportability by a mean of 32% and decreased viscoelasticity by a mean of 0.22 log units from the levels achieved with saline. The mixture of DA plus NAL at one-half of the above concentration of each agent produced an additional increase in the transportability, by a mean of 18%, and a further decrease in viscoelasticity, by a mean of 0.25 log units, compared with DA or NAL as a single treatment.

CONCLUSIONS: The combination of DA and NAL exhibits an additive effect for both the viscoelasticity and transportability of CF sputum samples. The two agents appear to act well together in breaking down the bonding due to extracellular DNA and mucins. Clinical studies should be undertaken to see whether the additive combination at lower concentration produces the anticipated benefits of improved airway clearance and fewer side effects.

Key Words: Cystic fibrosis; Mucociliary transport; Mucolytics; Sputum viscoelasticity

Résumé à la page suivante

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Effet additif de la dornase alfa et du nacystélyn sur la mobilisation et la viscoélasticité des expectorations dans le contexte de la mucoviscidose

OBJECTIF : Étudier l’effet de la dornase alfa (DA), du Nacystélyn (NAL) et de leur association sur la mobilisation des sécrétions mucociliaires et sur la viscoélasticité du mucus dans le contexte de la mucoviscidose (MV) et vérifier si leur association produit un effet additif.

PLAN D’ÉTUDE : Détérmination de la mobilisation des sécrétions dans des palais de grenouille et de la viscoélasticité in vitro.

PATIENTS : On a participé à l’étude 15 patients atteints de MV et présentant des infections chroniques à *Pseudomonas aeruginosa*.

INTERVENTIONS : Des prélèvements d’expectoration ont été mis en incubation, certains sans solution médicamenteuse servant de témoins, d’autres avec du sérum physiologique (SS), de la DA, du NAL et un mélange de DA et de NAL en des concentrations voisines de celles utilisées en clinique.

CONCLUSION : L’association de la DA et du NAL a eu un effet additif tant sur la viscoélasticité que sur la mobilisation des expectorations produites dans le contexte de la MV. Les deux agents semblent bien se compléter pour cliver les liaisons dues à l’ADN extracellulaire et aux mucines. Il faudrait menner des essais cliniques pour vérifier si l’effet additif de l’association à des concentrations plus faibles améliore toujours le dégagement des voies aériennes tout en produisant moins d’effets indésirables.

**Mucociliary transport (MT)** is a physiological response of the respiratory tract to clear both normal and excessive airway secretions (1). Impairment of MT, which may finally result in obstructive and damaged airways, is a characteristic feature of cystic fibrosis (CF). The decreased mucociliary transportability is primarily attributed to overproduction, accumulation and persistence of macromolecules (eg, DNA, albumin, etc) in the patient’s airways (2,3). The direct approach to increase mucociliary transportability of the CF gel mucus in airways is mucolysis, ie, the disruption of the gel network by altering the degree of crosslinking or the interactions between the macromolecules that form it (4). This disruption may be achieved through mucolytic agents such as dornase alfa (DA) (Pulmozyme, Genetech, USA) and Nacystelyn (NAL) (SMB & Galephar, Belgium) (4,5).

Because substantial amounts of DNA of high molecular weight are the leading cause of the tenacious and viscous properties of CF sputum (2,3), DA has been applied to treat CF sputum in vitro to hydrolyze the excess DNA in CF airway mucus and, thus, reduce the viscosity, changing it from rigid, poorly deformable material to a more fluid gel, thereby facilitating MT (2). Clinical studies have reported improved lung function in CF patients after DA treatment, and DA is widely used as a treatment for CF lung disease (6,7).

NAL is a compound of N-acetylcysteine (NAC) and L-lysine (8). It has been found in vitro to reduce the mucous gel disulfide bonds to sulfhydryl bonds, thereby reducing the mucoprotein viscosity and enhancing transportability (9). Preliminary clinical trials with NAL have shown it to be effective in reducing the viscoelasticity of sputum in CF patients (10).

Despite the many different mechanisms by which the rheological properties of mucus can be altered (7), very few studies have been carried out regarding the additive effects of mucolytics on CF sputum. Our previous experiments demonstrated that the combination of DA and NAL at one-half the concentration of each agent significantly decreased the spinnability (one of the rheological parameters) of CF sputum more than either treatment by itself (4). We hypothesized that their combination would also improve viscoelasticity (the most important rheological factor) and mucociliary transportability more than either of them on their own.

To investigate this hypothesis, the researchers measured and compared in vitro the viscoelasticity (log G*) of CF sputum before and after treatment with DA, NAL and their combination. In the present study, the authors also employed the mucus depleted frog palate as the animal model to determine mucociliary transport velocity (MTV), a direct index of mucociliary transportability of CF patient sputum.

**MATERIALS AND METHODS**

**Subjects**

Sputum samples were collected from 15 patients with CF by voluntary expectoration during a routine clinical visit before a clinical trial of azithromycin. The patients, aged 10 to 19 years, were all infected with *Pseudomonas aeruginosa*. Eight of the patients were receiving oral NAC, usually 600 mg/day. Four of these eight patients were also receiving a daily inhalation of 2.5 mg DA. In most cases, the patient sputum was collected in the morning, before the administration of any mucolytic medication. Collection and use of sputum for this experiment were...
The study design, the Viscoelasticity measurements, and the MTV determination using frog palates are described in the text. A figure illustrating the additive effect of dornase alfa and Nacystelyn on CF sputum is included. The statistical analysis of the results is also discussed.
RESULTS

Ciliary transportability

Saline treatment produced a small but statistically significant increase in MTV from the untreated control (17.87±0.98 mm/min versus 16.35±0.91 mm/min) (Figure 1). The application of DA to the sputum (5 µg/g final concentration) produced a significant increase in MTV compared with incubation with normal saline (23.42±1.41 versus 17.87±0.98 mm/min). The application of 100 µM of NAL also increased MTV significantly more than normal saline (23.02±1.37 versus 17.87±0.98). There was no significant difference in MTV between the application of DA and NAL at the indicated concentrations.

Compared with treatment with normal saline, the combined treatment of DA and NAL (one-half the above mentioned concentration of each mucolytic agent) produced a significant increase in MTV in MTV (26.27±2.07 versus 17.87±0.98) (Figure 1). The combined treatment also significantly increased MTV compared with the application of either DA or NAL alone.

Sputum viscoelasticity

Table 1 shows the viscoelastic data, presented as log G* (mean ± SE) for 15 samples before and after different treatments. Figure 2 shows the values expressed as the change in log G* value (% control linear scale) from the pretreatment control values and the statistical significance between the different groups. Incubation only (untreated control) resulted in a slight decrease (0.073 log units, P=0.0395) from the pretreatment viscoelasticity. Saline treatment (incubation plus dilution) resulted in a further modest decrease in log G* (0.296 units, P=0.0105). DA treatment at 5 µg/g final concentration (approximately 200 nM) decreased the viscoelasticity by a mean of 0.60 log units (to approximately 25% control, P=0.0127). NAL treatment at 100 µM decreased the viscoelasticity by a similar amount (0.518 log units, P=0.0522 with respect to saline).

The combination DA plus NAL at one-half the concentration of each agent resulted in the greatest decrease in viscoelasticity (0.813 log units, approximately 15% control, P=0.0001). The decrease in viscoelasticity due to DA plus NAL was significantly greater than for NAL alone (P=0.012) but not for DA alone (P=0.0735).

Both components of G* (G′ – elasticity and G″ – viscosity) decreased with mucolytic treatment. There was no significant change in tan δ (G′/G″).

The response to in vitro mucolytic treatment (decrease in viscoelasticity compared with vehicle) was not different in those patients who had been receiving mucolytic drugs (NAC or DA) versus those who did not (Table 2). This was also the case in terms of in vitro ciliary transportability.

DISCUSSION

CF sputum treated with either DA (200 nM) or NAL (100 µM) demonstrated a significant increase in transportability and a significant decrease in viscoelasticity compared with CF sputum treated with saline. However, in comparison with singular treatments, the combined treatment of DA and NAL, at one-half the concentration of each mucolytic agent, showed an even larger, statistically significant increase in transportability and decrease in viscoelasticity of CF sputum. This finding is important because it indicates that the two agents clearly complement one another and, importantly, do not interfere with each other’s action.

The enzyme DA has the ability to break down large concentrations of DNA, thus improving the rheological properties of CF sputum. In this study, CF sputum treated with DA at a final concentration of 5 µg/l demonstrated an
higher than those used in the present investigation. were more responsive to DA than to NAL; although in the by Shah et al (20), who reported that CF sputum samples of CF sputum with DA concurs with the results presented of equipotency. This tendency to greater rheological effect approximately six times as great as that of DA at the point mucolytic concentration used in our study, which was 6.4-fold greater than DA, and thus, likely relevant to the Belgium, private communication). This dose is nominally the clinic (19). The current dose of NAL in clinical devel-

The DA dose pertains to 2 to 4 µg/mL of DA in CF sputum, terms of rheological and transport effects) to 5 µg/g of DA. The dose of NAL at 31 µg/g has equivalence (in manifest significant effects on the viscoelasticity of CF sputum. The dose of NAL is achieved by aerosol of DA at a dose of 2.5 mg in the clinic (19). The current dose of NAL in clinical development is 16 mg by dry powder inhaler (SMB & Galephar, Belgium, private communication). This dose is nominally 6.4-fold greater than DA, and thus, likely relevant to the mucolytic concentration used in our study, which was approximately six times as great as that of DA at the point of equipotency. This tendency to greater rheological effect of CF sputum with DA concurs with the results presented by Shah et al (20), who reported that CF sputum samples were more responsive to DA than to NAL; although in the latter study, the concentration of both drugs were much higher than those used in the present investigation.

NAL is a derivative of NAC, a thiol-reducing agent that breaks disulfide bonds. NAC has been widely used to treat MT disorders (15). Previous studies have shown that NAL has greater mucolytic activity than NAC (16, 17) and preliminary clinical trials with NAL have shown it to be effective in reducing the viscoelasticity of sputum in CF patients (10). Marriott et al (16), using porcine gastric mucus, found significant mucolytic activity with NAL starting at 8 µM concentration, while App et al (17) also found a significant activity at 10 µM in sputum, which is lower than the concentration used in the present study. Other thiol-reducing agents, such as dithiothreitol or mercaptoethane sulfonate, may overliquefy mucus (17), thereby making it unsuitable for clearance by ciliary action (18).

In the present study, two different doses of NAL and DA manifested significant effects on the viscoelasticity of CF sputum. The dose of NAL at 31 µg/g has equivalence (in terms of rheological and transport effects) to 5 µg/g of DA. The DA dose pertains to 2 to 4 µg/mL of DA in CF sputum, which is achieved by aerosol of DA at a dose of 2.5 mg in the clinic (19). The current dose of NAL in clinical development is 16 mg by dry powder inhaler (SMB & Galephar, Belgium, private communication). This dose is nominally 6.4-fold greater than DA, and thus, likely relevant to the mucolytic concentration used in our study, which was approximately six times as great as that of DA at the point of equipotency. This tendency to greater rheological effect of CF sputum with DA concurs with the results presented by Shah et al (20), who reported that CF sputum samples were more responsive to DA than to NAL; although in the latter study, the concentration of both drugs were much higher than those used in the present investigation.

The combined treatment of sputum with DA and NAL (at one-half the concentration of each mucolytic agent) increased transportability and decreased viscoelasticity significantly more than the singular treatments with either DA or NAL. Although synergism was not formally tested by studying the concentration dependence of the mucolytic behaviour, it is clear from previous studies (4,17) that reducing the concentration of either DA or NAL would have resulted in a decrease in viscoelastic effect. Thus, it is reasonable to describe the supra-additivity of mucolytic activity as an additive effect.

This additive behaviour of DA and NAL in increasing transportability and decreasing viscoelasticity of CF sputum may be due, in part, to cooperative rearrangements of the bonding and intermolecular interactions between neighbouring molecules. Reduction of the mucin disulfide bonds to sulfhydryl bonds by NAL may make DNA more accessible for action by DA. At the same time, cleavage of high molecular weight DNA by DA may assist in increasing the sputum transportability by NAL. These favourable alterations in sputum rheological properties would predict improved expectoration (18) and, ultimately, an improvement in lung function of CF patients, provided that overliquefication of sputum does not occur. Although reduction of the disulfide bonds in DA by NAL, and thus interference with its DA action, is a theoretical possibility, there is no suggestion from our findings that this might have occurred. DA should have little effect on normal mucin networks, as suggested by rheological experiments using tracheal mucus from healthy dogs (21).

CONCLUSIONS

The results of the present studies both in frog palate and in vitro suggest that the combined treatment of CF sputum with DA and NAL may lessen the respiratory burden caused by the impaired clearance of airway secretions with abnormal rheological properties in these patients. By combining DA and NAL, mucolysis may not only be more appropriate for rheological change, but also more cost effective in the treating disorders of impaired MT of sputum with abnormal rheological properties. NAL has not yet been tested clinically in North America. Because NAL appears to work in vitro at lower concentration than its par-

### TABLE 2

<table>
<thead>
<tr>
<th>TABLE 2</th>
<th>Decrease in viscoelasticity (log G* at 10 rad/s) and increase in mucociliary transport velocity (MTV) of cystic fibrosis sputum of patients who had received mucolytic drugs versus those who had not</th>
<th>Additive effect of dornase alfa and Nacystelyn on CF sputum</th>
</tr>
</thead>
<tbody>
<tr>
<td>dlog G* at 10 rad/s</td>
<td>Control</td>
<td>DA</td>
</tr>
<tr>
<td>No mucolytics (n=7)</td>
<td>0.140±0.08</td>
<td>0.246±0.07</td>
</tr>
<tr>
<td>Mucolytics (n=8)</td>
<td>0.014±0.06</td>
<td>0.340±0.06</td>
</tr>
<tr>
<td>MTV (% control)</td>
<td>Saline</td>
<td>DA</td>
</tr>
<tr>
<td>No mucolytics (n=3)</td>
<td>109.3±0.7</td>
<td>141.3±15.7</td>
</tr>
<tr>
<td>Mucolytics (n=4)</td>
<td>109.5±0.6</td>
<td>145.8±5.7</td>
</tr>
</tbody>
</table>

Additives of CF sputum samples were incubated without treatment (control), or with saline, dornase alfa (DA, 200 nM), Nacystelyn (NAL, 100 µM) (SMB & Galephar, Belgium) or DA+NAL (100 nM / 50 µM) at 37°C for 30 min. dlogG* and MTV data are presented as means plus or minus standard errors for the number of samples indicated.

increase in transportability. This result is similar to that reported by Zahn et al (14). They observed improvements in sputum transportability (by frog palate assay) with DA at concentrations ranging from 0.2 to 20 µg/mL. In addition, DA at a concentration of 200 nM was found to decrease the sputum viscoelasticity in vitro. These data may explain our previous experimental results that DA at a concentration of 100 nM (lower than the present one) succeeded in decreasing the spinnability of CF sputum but failed to decrease the viscoelasticity (4).

Mucolytics (n=8) 109.5±0.6 145.8±5.7 141.7±4.6 165.9±7.0
ent compound NAC, and its pH is more neutral, the potential for side effects is reduced. Furthermore, combining it with another mucolytic approach, as the results of the present study suggest, could minimize such undesirable effects. It is possible, however, that NAL interacts with DA molecules, leading to either a reduction in its hydrolytic activity or alteration in its conformation. Thus, data on drug interaction are needed before conducting further studies on patients. Additional studies with experiments in a large patient population are required to confirm these findings and investigate issues regarding the safety and efficacy of long term administration of DA and NAL in combination.

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REFERENCES
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