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ORIGINAL RESEARCH

Hyaluronic Acid Improves "Pleasantness" and Tolerability of Nebulized Hypertonic Saline in a Cohort of Patients with Cystic Fibrosis

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ABSTRACT

Introduction: Inhaled hypertonic saline improves lung function and decreases pulmonary exacerbations in people with cystic fibrosis. However, side effects such as cough, narrowing of airways and saltiness cause intolerance of the therapy in 8% of patients. The aim of our study was to compare the effect of an inhaled solution of hyaluronic acid and hypertonic saline with hypertonic solution alone on safety and tolerability. ***Methods:*** A total of 20 patients with cystic fibrosis aged 6 years and over received a single treatment regimen of 7% hypertonic saline solution or hypertonic solution with 0.1% hyaluronate for 2 days nonconsecutively after a washout period in an open crossover study. Cough, throat irritation, and salty taste were evaluated by a modified ordinal score for assessing tolerability; "pleasantness" was evaluated by a five-level, Likert-type scale. Forced expiratory volume in 1 second was registered before and after the end of the saline inhalations. ***Results:*** All 20 patients (nine males, 11 females, mean age 13 years, range 8.9-17.7) completed the study. The inhaled solution of 0.1% hyaluronic acid and hypertonic saline significantly improved tolerability and pleasantness compared to hypertonic saline alone. No major adverse effects were observed. No difference was documented in pulmonary function tests between the two treatments. ***Conclusion:*** Hyaluronic acid combined with hypertonic saline solution may contribute to improved adherence to hypertonic saline therapy. Further clinical trials are needed to confirm our findings. Considering the extraordinary versatility of hyaluronic acid in biological reactions, perspective studies could define its applicability to halting progression of lung disease in cystic fibrosis.

Keywords: cystic fibrosis; hyaluronic acid; hypertonic saline solution; pleasantness; tolerability

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INTRODUCTION

Cystic fibrosis (CF) is caused by mutations in the gene encoding the CF transmembrane

conductance regulator (CFTR), an anion channel expressed in epithelial cells throughout the body. CF lung disease is the major cause of morbidity and mortality in CF despite the many therapies aimed at remedying it. In the lung, as a consequence of CFTR dysfunction, unrestrained Na^+ absorption and failure of active Cl^- secretion leads to a decreased airway surface liquid (ASL) volume and subsequent abnormal mucociliary clearance.[1-3] Mucous retention favors bacterial infection and inflammation, leading to lung damage and ultimately respiratory failure.

Increasing evidence supports the hypothesis that lung disease in CF reflects the vulnerability of the airway surface to dehydration. Trials investigating the potential of hypertonic saline solution (HS) on accelerating mucociliary clearance, as well as expanding ASL, have been recently proposed as a new option to increase ASL hydration in patients with CF.[4-6] Sufficient evidence has been collected to recommend inhaled HS as an alternative mucolitic agent in CF, in order to improve quality of life and reduce pulmonary exacerbations.[7-9]

Although HS treatment is well tolerated by the majority of patients, some adverse events such as cough, narrowing of the airways, and unpleasant salty taste are described, limiting its application despite pretreatment with inhaled bronchodilatator. Side effects may also lead to poor adherence and cause patient dropout from the treatment in about 10% of cases.[10]

In recent years, new insights have been gained into the functional role of matrix components modulating lung injury. One of the most interesting matrix components is hyaluronic acid (HA). HA is a natural polysaccharide containing a repeated series of disaccharide units of glucuronic acid and *N*-acetylglucosamine that occurs in many tissues and body fluids in vertebrates. HA belongs to a family of structurally similar polysaccharides called glycosaminoglycans. It has several physiological functions, such as a barrier effect and water homeostasis in the interfibrillar spaces, contributing to constitute the fundamental part of the amorphous colloidal matrix, and to determine relevant effects on tissue morphogenesis through interaction with a number of widespread HA-binding proteins.[11] In particular, HA functions as a major matrix substance in which cells and fibrous constituents of the matrix such as elastin and collagen are embedded. It prevents elastin damage by elastases and modulates neutrophil elastase secretion, as has been reported in animal models.[12]

HA exists in solution in a flexible, coiled configuration, is well hydrated and contains approximately 1000-fold more water than polymer.[13] The molecular weight of HA varies in specific tissues. Normal synovial fluid has HA of an average of 7000 kDa,[14] whereas in cartilage and the lung it is 2000[15] and 220 kDa,[16] respectively. In the normal human adult lung, the total HA content is approximately 160 mg. The most likely major role of HA is the regulation of fluid balance in the interstitium through its high water-binding capacity, as noted in both fetal rabbit and human adult lungs, facilitating ventilation and gas exchange.[17]

From the perspective of previous data, there is evidence that HA may exert a protective effect against injury in a number of respiratory diseases.[18] While a predicted role of HA has been documented in experimental animal models of lung emphysema and chronic obstructive pulmonary disease,[12] no safety data are available with regard to its therapeutic potential in lung diseases in humans.

Venge and colleagues[19] demonstrated that subcutaneous HA administration significantly reduced the number of bacterial infections in patients with chronic bronchitis compared to placebo-treated patients, possibly due to enhancing cellular host defense mechanisms.

In a randomized, crossover, single-blind study design,[20] aerosol HA administration significantly reduced the bronchial hyper-reactivity to muscular exercise in 14 patients, all suffering from mild bronchial asthma. Pretreatment with HA determined a partial but clear-cut protection of the bronchoconstrictive effect induced by the muscular exercise. Such effects could be attributed to the correction of loose connective amorphous matrix of the airways, one of the main features involved in the pathological remodeling of asthma. However, other data did not confirm these results.[21] No previous study has evaluated the possible role of HA as a mucolitic therapeutic strategy in CF.

Despite the well-known beneficial effects of long-term use of HS on a number of exacerbations, quality of life and absenteeism, HS safety and tolerability should be ameliorated in order to avoid poor compliance to therapy. Our preliminary work for the first time explores the hypothesis that inhaled HS with HA could improve the tolerance and "pleasantness" (as compared to the perceived pleasantness of HS without HA) of HS, reducing the dropout from this treatment.

MATERIALS AND METHODS

Study Subjects

Participants were recruited from a regional pediatric CF center that is responsible for 190 patients. All patients attending the clinic during the recruitment period were consecutively approached and informed about the study. After willingness and eligibility were confirmed, participants were formally enrolled in the study. Written informed consent was obtained for each patient from guardians. Inclusion criteria for the study were: an established diagnosis of CF, age at least 6 years, a forced expiratory volume in 1 second (FEV_1) of 50% or more of the predicted value, and clinically stable lung disease. CF diagnosis was confirmed by sweat testing (chloride ≥60 mmol/L by quantitative pilocarpine iontophoresis) and identification of two well-characterized genetic CFTR mutations. Exclusion criteria were: evidence of reactive airways or a clinical diagnosis of asthma. The study was approved by the local ethics committee.

Study Design

The study design was an open, randomized, crossover trial comparing a single treatment of HS on 1 day and a single treatment of HS with HA on another day, with a washout period of 1 day. Recruitment period was 1 month. Participants were randomly assigned a treatment order using computerized randomization, with a 1:1 ratio. Allocation was concealed using an investigator who was off site. The primary outcome of the study was to assess the tolerability of the inhaled solution due to cough, because this is the most common cause of intolerance of HS therapy. Secondary outcomes included tolerability of the inhaled solution in terms of throat irritation and salty taste, and the perceived pleasantness of the inhalation. Other major adverse events such as bronchospasm and hemoptysis were also noted. Details of the study design are reported in Figure 1. At baseline, age, height, and gender were recorded to allow the calculation of lung function values predicted by normative equations (Table 1). Lung function testing was performed according to standardized criteria.

Interventions

Salbutamol at a dose of 200 µg was administered 30 minutes before each inhalation of the study medication. When participants

Table 1. Characteristics of participants.

Characteristic	Included participants (*n*=20)
Age in years, mean (range)	13 (8.9-17.7)
Height in cm, mean (±SD)	154 (±15.1)
BMI in kg/m^2, mean (±SD)	19.49 (±2.43)
Male, *n* (%)	9 (45)
FEV_1 % predicted, mean (range)	91 (53-130)
Pancreatic insufficiency, *n* (%)	18 (90)
Genotype ΔF508/ΔF508, *n* (%)	8 (40)
Genotype ΔF508/other, *n* (%)	8 (40)
Genotype other/other, *n* (%)	4 (20)

Pancreatic insufficiency was determined by fecal elastase 1 below 100 μg/g feces.
% predicted=percentage of predicted value for age, height, and gender.
BMI=body mass index; FEV_1=forced expiratory volume in 1 second.

were randomly assigned to receive HS, they inhaled one 5 mL dose of 7% sodium chloride solution. When they were randomly assigned to receive HS+HA, they inhaled one 5 mL dose of 7% sodium chloride solution with 0.1% HA. This concentration of HA is the highest dose permitted for administration to humans in clinical research by the US Food and Drug Administration, and it is easily and comfortably inhaled.[13] Delivery of both saline solutions was via a Pari LC plus nebulizer and a Pari Proneb Ultra compressor (Pari-Pharma GmbH, Starnberg, Germany). During the study, all participants performed airway clearance physiotherapy using a positive expiratory pressure mask, according to a standard treatment protocol.[22]

Outcome Measures

The primary outcome was the tolerability of the inhaled solution due to cough. Tolerability of the inhaled solution was evaluated in terms of throat irritation, salty taste, and the perceived pleasantness of the inhalation. Immediately after each inhaled dose patients registered symptoms on a diary card. Participants rated the severity of their symptoms including cough, irritation (burning in the throat), and saltiness, scored by a four-point ordinal scale where 0=absent and 3=severe. Pleasantness was evaluated using a standardized five-level, Likert-type scale where 1=very unpleasant and 5=very pleasant. Participants performed standardized spirometry before and after each saline inhalation.

The FEV_1 test was performed immediately before and after the dose of each treatment. Other major adverse events were also noted.

Data Analysis

The primary outcome of cough was treated as a dichotomous variable (present vs. absent) and compared between groups using McNemar's exact chi-square test with a 95% confidence interval. The same test was also used to compare the severity of cough as the primary outcome. McNemar's test was also used to compare the presence of throat irritation between groups and the presence of salty taste between groups. The Wilcoxon paired-sample test was used to compare the pleasantness of the inhalation between groups. Significance was set at $P<0.05$.

Sample Size Calculation

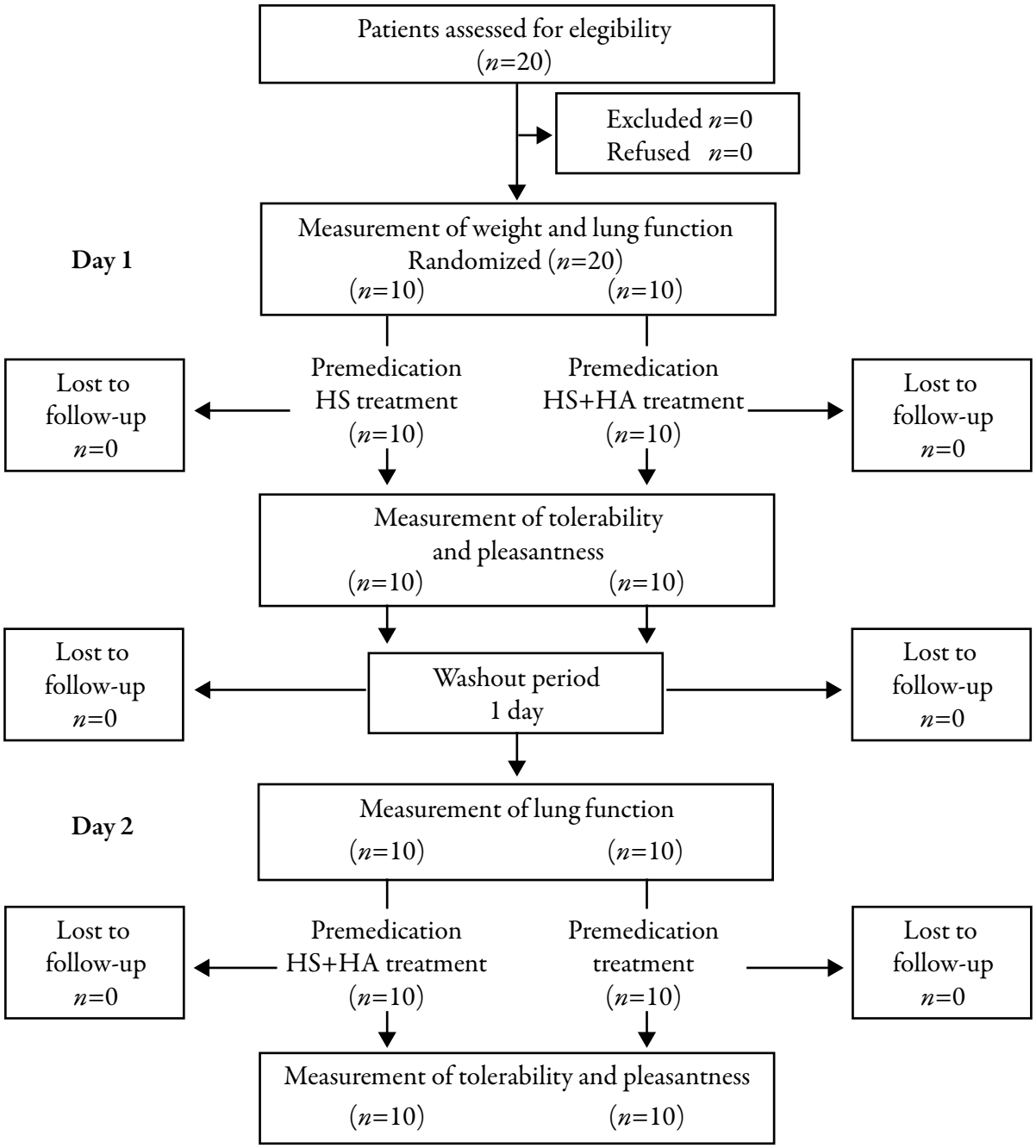
Approximately 90% of patients with CF cough during inhalation of HS. In the absence of an established minimum clinically important difference in cough, we decided that if the prevalence of cough after saline inhalation were reduced by half, this would represent a clinically worthwhile reduction. Assuming a correlation coefficient between paired subjects of 0.2, a total of 20 patients would provide 80% probability of detecting this difference in cough prevalence at a two-sided 1% significance level.

RESULTS

In all, 20 participants (nine males, 11 females mean age 13 years, range 8.9-17.7) were recruited and underwent baseline testing. All participants had routinely received 7% HS. Randomization was used to allocate 10 patients to each treatment order. All participants completed the interventions as allocated and all completed postintervention testing, as shown in Figure 1.

For each symptom measure (cough, throat irritation, salty taste) and for pleasantness, a

Figure 1. Flow of participants through the trial. HA=hyaluronic acid; HS=hypertonic saline.



statistically significant difference was found between participants treated with HS+HA versus HS alone (P<0.05). Data are shown in Figures 2 and 3.

Figure 2. Severity of (**A**) cough, (**B**) throat irritation, and (**C**) salty taste after hypertonic saline inhalation versus inhalation of hypertonic saline with hyaluronic acid, expressed as a percentage of studied patients. A=hypertonic saline inhalation. B=hypertonic saline with hyaluronic acid.

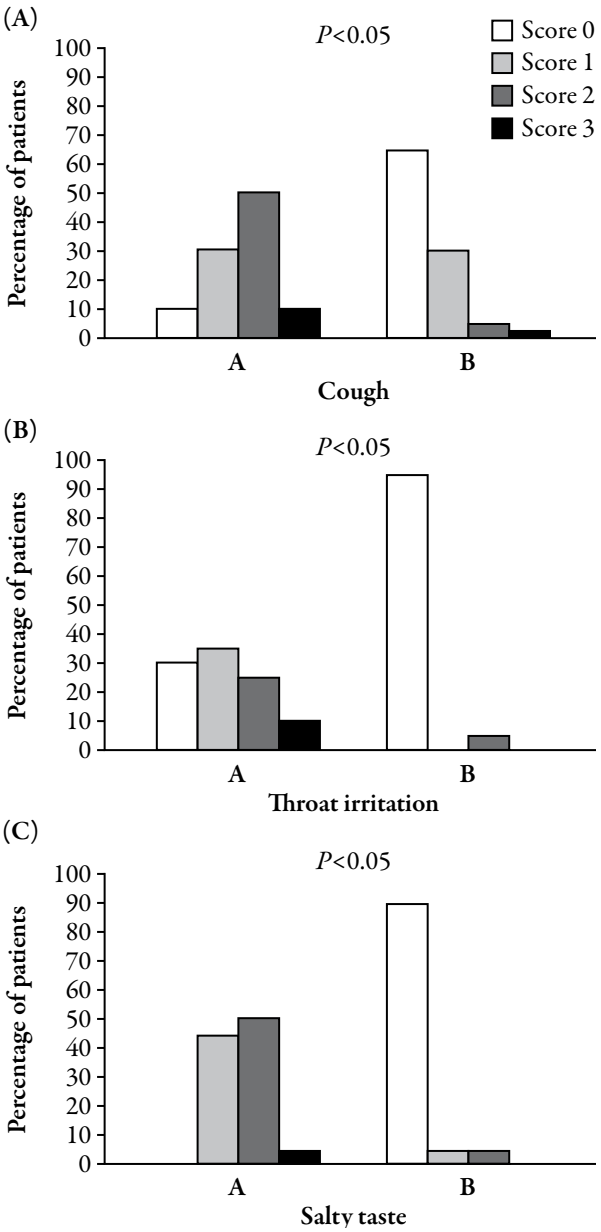
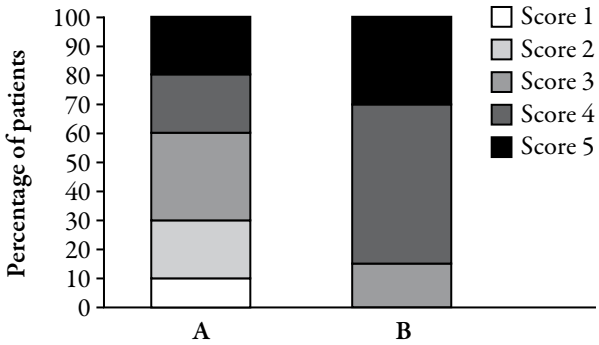


Figure 3. Rating of "pleasantness" after hypertonic saline inhalation (**A**) versus inhalation of hypertonic saline with hyaluronic acid (**B**), expressed as a percentage of studied patients.



Cough

Cough occurred immediately after inhalation of HS in 18 of 20 participants (90%). Cough occurred immediately after inhalation of HS+HA in 7 of 20 participants (35%). The addition of HA statistically significantly reduced the prevalence of cough immediately after inhalation (absolute risk reduction 55%; 95% CI: 25%, 73%). The severity of the cough experienced was statistically significantly less when the HS contained HA, with a median severity of 2 (moderate) for HS and 0 (absent) for HS+HA (P<0.05) (Figure 2A).

Throat Irritation

Throat irritation occurred immediately after inhalation of HS in 14 of 20 participants (70%). Throat irritation occurred immediately after inhalation of HS+HA in 1 of 20 participants (5%). The addition of HA statistically significantly reduced the prevalence of throat irritation immediately after inhalation (absolute risk reduction 65%; 95% CI: 36%, 81%). The severity of the throat irritation experienced was statistically significantly less when the HS contained HA, with a median severity of 1 (mild) for HS and 0 (absent) for HS+HA (P<0.05) (Figure 2B).

Salty Taste

A salty taste was reported immediately after inhalation of HS by all participants, but only by 2 of 20 participants (10%) after inhalation of HS+HA. The addition of HA statistically significantly reduced the prevalence of salty taste immediately after inhalation (absolute risk reduction 90%; 95% CI: 64%, 97%). The severity of the salty taste experienced was statistically significantly less when the HS contained HA, with a median severity of 2 (moderate) for HS and 0 (absent) for HS+HA ($P<0.05$) (Figure 2C).

Pleasantness

Participants' ratings of pleasantness showed that the addition of HA to HS resulted in significantly more pleasant ratings. The rating of pleasantness of the taste experienced was statistically significantly less when the HS contained HA, with a median severity of 2 (moderate) for HS and 0 (absent) for HS+HA ($P<0.05$) (Figure 3).

Other Outcomes

Mean values of FEV_1 for the study population at baseline was 91.45% (range 53%-130%).

FEV_1 from preinhalation to postinhalation of HS was identical to FEV_1 preinhalation to postinhalation of HS+HA. There were no major adverse events on either day.

DISCUSSION

In considering the validity and applicability of the results of this study, several points should be noted. The study reached its intended sample size, due to the statistic analysis, and there were no dropouts or missing data. Also, there were no refusals among the consecutive cohort of potential participants who were invited to join the study. The study clearly achieved its objective of determining whether HA improves the tolerability and pleasantness of HS inhalations, since previous data report that an important proportion of patients cannot tolerate HS long-term therapy, most commonly due to excessive cough. Although excessive coughing can be an unwanted side effect, cough is one of several important mechanisms by which HS improves mucociliary clearance and clinical outcomes. Since no difference was found in 24-hour sputum volume between five patients of each treated group (data not shown), it is reassuring that the reduction in coughing by HA does not modify the effect of HS on sputum clearance.

Our study is limited by several caveats. Randomized controlled trials should be carried out to support our hypothesis. Nevertheless, the crossover design allowed us to avoid selection bias. Limitations could also be related to the format used for measuring self-efficacy. The Likert scale has been used in several studies as an alternative measurement format to the traditional format for measuring self-efficacy, as reported previously. Previous data indicate that Likert-type and traditional measures of self-efficacy have similar reliability.[23] In our study the use of the Likert-type scale allowed us to set selected questions, easy for patients to understand, in order to lead consistent answers.

Since statistically significant benefits of HA were identified in the presence of the three symptoms assessed and in the participants' subjective ratings of the pleasantness of the inhalation, we suggest that HA facilitates use of HS therapy in patients with CF. As discussed, it may allow tolerance of the therapy in intolerant patients, as well as improving adherence to regular inhalations even in patients who currently tolerate the therapy. This would, in turn, improve the outcomes of the therapy.

Furthermore, we reviewed the evidence that HA may have a protective effect against lung injury by common processes in CF lung disease. Therefore, long-term studies are indicated in order to evaluate the efficacy of this new formulation. Such trials should also consider the cost-effectiveness of HS+HA as an alternative to current mucolytic therapy such as recombinant human DNase.

CONCLUSION

HA significantly improves the tolerability and pleasantness of HS in patients with CF. Larger longitudinal controlled trials are needed to confirm the clinical efficacy of this intervention on pulmonary function, pulmonary exacerbations and ultimately quality of life. Further studies are needed to establish whether these results are also applicable to severely affected patients with CF.

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REFERENCES

1. O'Sullivan BP, Freedman SD. Cystic fibrosis. Lancet. 2009;373:1891-1904.
2. Collins FS. Cystic fibrosis: molecular biology and therapeutic implications. Science. 1992;256:774-779.
3. Welsh MJ, Smith AE. Molecular mechanisms of CFTR chloride channel dysfunction in cystic fibrosis. Cell. 1993;73:1251-1254.
4. Boucher RC. Evidence for airway surface dehydration as the initiating event in CF airway disease. J Intern Med. 2007;261:5-16.
5. Elkins MR, Robinson M, Rose BR, et al. A controlled trial of long-term inhaled hypertonic saline in patients with cystic fibrosis. N Engl J Med. 2006;354:229-240.
6. Subbarao P, Balkovec S, Solomon M, Ratjen F. Pilot study of safety and tolerability of inhaled hypertonic saline in infants with cystic fibrosis. Pediatr Pulmonol. 2007;42:471-476.
7. Flume PA, O'Sullivan BP, Robinson KA, et al. Cystic fibrosis pulmonary guidelines: chronic medications for maintenance of lung health. Am J Respir Crit Care Med. 2007;176:957-969.
8. Donaldson SH, Bennett WD, Zeman KL, Knowles MR, Tarran R, Boucher RC. Mucus clearance and lung function in cystic fibrosis with hypertonic saline. N Engl J Med. 2006;354:241-250.
9. Wark P, McDonald VM. Nebulised hypertonic saline for cystic fibrosis. Cochrane Database Syst Rev. 2009;15:CD001506.
10. Elkins MR, Bye PT. Inhaled hypertonic saline as a therapy for cystic fibrosis. Curr Opin Pulm Med. 2006;12:445-452.
11. Rosenberg RC, Varma R. An overview of proteoglycans in physiology and pathology. In: Varma RS, Varma R, eds. Glycosaminoglycans and proteoglycans in physiological and pathological processes of body systems. Basel: Karger; 1982;1-4.
12. Scuri M, Abraham WM. Hyaluronan blocks human neutrophil elastase (HNE)-induced airway responses in sheep. Pulm Pharmacol Ther. 2003;16:335-340.
13. Laurent TC. Structure of hyaluronic acid. In: Balazs EA, ed. Chemistry and molecular biology of the intercellular matrix. London: Academic Press; 1970;703-732.
14. Fraser JRE, Laurent TC, Laurent UBG. Hyaluronan: its nature, distribution, functions and turnover. J Intern Med. 1997;242:27-33.
15. Holmes MWA, Bayliss MT, Muir H. Hyaluronic acid in human articular cartilage. Biochem J. 1988;250:435-441.
16. Nettelbladt O, Tengblad A, Hallgren R. Lung accumulation of hyaluronan parallels pulmonary edema in experimental alveolitis. Am J Physiol. 1989;257:L379-L384.
17. Schmid K, Grundboeck-Jusco J, Kimura A, et al. The distribution of the glycosamino- glycans in the anatomic components of the lung and the changes

in concentration of these macromolecules during development and aging. Biochim Biophys Acta. 1982;716:178-187.

18. Turino GM, Cantor O. Hyaluronan in respiratory injury and repair. Am J Respir Crit Care Med. 2003;167:1169-1175.

19. Venge P, Pedersen B, Hakansson L, Hallgren R, Lindblad G, Dahl R. Subcutaneous administration of hyaluronan reduces the number of infectious exacerbations in patients with chronic bronchitis. Am J Respir Crit Care Med. 1996;153:312-316.

20. Petrigni G, Allegra L. Aerosolised hyaluronic acid prevents exercise-induced bronchoconstrinction, suggesting novel hypotheses on the correction of matrix defects in asthma. Pul Pharmacol Ther. 2006;19:166-171.

21. Kunz LI, van Rensen EL, Sterk PJ. Inhaled hyaluronic acid against exercise-induced bronchoconstriction in asthma. Pulm Pharmacol Ther. 2006;19:286-291.

22. McIlwaine PM, Wong LT, Peacock D, Davidson AG. Long-term comparative trial of conventional postural drainage and percussion versus positive expiratory pressure physiotherapy in the treatment of cystic fibrosis. J Pediatr. 1997;131:570-574.

23. Maurer JT, Pierce HR. A comparison of Likert scale and traditional measures of self-efficacy. J Appl Psychol. 1998;83:324-349.