

## Short-Term Efficacy of Ultrasonically Nebulized Hypertonic Saline in Cystic Fibrosis

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**Summary.** Progressive lung disease in patients with cystic fibrosis (CF) is caused by thick secretions, which cause airway obstruction and subsequent colonization and infection by inhaled pathogenic microorganisms. Recently, recombinant human DNase has been shown to reduce the viscoelasticity of sputum in patients with cystic fibrosis and to improve lung function. Ultrasonically nebulized hypertonic saline (HS) has been demonstrated to enhance mucociliary clearance and sputum expectoration by rehydrating airway secretions, and may therefore provide a low cost alternative. We studied the changes in pulmonary function and symptoms in a group of patients with CF who have moderate to severe lung disease. The patients were evaluated following 2 weeks of treatment with HS in an open-label study. Subjects were randomly allocated to receive 10 ml of either 0.9% NaCl (IS) or 6% NaCl (HS). Twice daily, prior to physiotherapy, treatments were delivered by a portable ultrasonic nebulizer. To prevent bronchoconstriction, 600 mg of salbutamol was administered prior to the nebulized solutions. A symptom score was recorded and spirometry was performed on day 0 before therapy was started, on day 14 (the last day of therapy), and on day 28 (14 days after the last treatment with either IS or HS). Fifty-two patients (32 males), with a mean age of 16.2 (range 7–36) years completed the study. There was no difference in baseline characteristics between the two groups. Following 2 weeks of treatment, there was a significant improvement from baseline in FEV<sub>1</sub> of  $15.0 \pm 16.0\%$  (mean  $\pm$  SD) in patients treated with HS, compared with a change of  $2.8 \pm 13\%$  in those on IS therapy ( $P = 0.004$ ). Furthermore, there was a subjective improvement in the effectiveness of chest physiotherapy as reported by those using HS ( $P = 0.02$ ). The treatment was well tolerated. *We conclude* that in patients with CF, ultrasonically nebulized hypertonic saline improves lung function in a way similar to that reported for human recombinant DNase when inhaled over a 2 week period. Nebulized saline also enhances the perception of effectiveness of chest physiotherapy. *Pediatr Pulmonol.* 1996; 21:77–83. © 1996 Wiley-Liss, Inc.

**Key words:** Hypertonic saline, cystic fibrosis, mucolytics.

### INTRODUCTION

Cystic fibrosis (CF) is the most common autosomal recessive disease in the Caucasian population, with an estimated incidence of 1 in 2,500.<sup>1</sup> Although CF is a generalized disorder of exocrine glands, progressive suppurative lung disease accounts for most of the morbidity and mortality associated with the disease.<sup>2</sup> Factors that contribute to the progression of lung disease include the increased viscoelasticity of the sputum and relative dehydration of the periciliary fluid, both of which inhibit mucociliary clearance.<sup>3</sup> Retention of abnormal airway secretions promotes infection and the progressive destruction of airways and adjacent lung tissue.<sup>4,5</sup>

In recent years, biotechnology has produced recombinant human DNase. The tenacity and viscosity of sputum

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in patients with CF is, in part, a consequence of large amounts of DNA released by dead neutrophils. Aerosolized DNase is thought to cleave DNA into shorter segments, thus facilitating expectoration. Placebo-controlled clinical trials have shown that regular treatment with aerosolized recombinant human DNase improves lung function and reduces the frequency of respiratory tract infections.<sup>6,7</sup>

A number of approaches have been taken to correct the disordered electrolyte and water content of the airway surface liquid. In CF, a defect in the control of Cl<sup>-</sup> channels on the apical surface of the respiratory epithelium results in reduced chloride secretion and excessive absorption of sodium ions and water.<sup>8-10</sup> Amiloride, applied to the luminal surface of respiratory epithelium is able to correct the increased transepithelial potential difference in patients with CF.<sup>11</sup> Regular inhalation of amiloride has been shown to improve mucociliary and cough clearance in patients with CF<sup>12</sup>, however, the addition of inhaled amiloride to treatment with bronchodilators and/or antibiotics does not result in any demonstrable improvement in lung function when compared with placebo.<sup>13</sup>

The application of hypertonic saline (HS) to the surface of the respiratory epithelium increases the amount of Na<sup>+</sup> and Cl<sup>-</sup> in airway surface liquid. As a result, the osmotic gradient is increased and water is attracted back onto the airway surface, thus rehydrating the periciliary fluid.<sup>14</sup> Inhalation of aerosolized HS has been shown to enhance mucociliary clearance in CF<sup>15</sup> and to improve sputum expectoration.<sup>16</sup> The aim of this study was to determine whether the regular inhalation of ultrasonically nebulized HS over a 2 week period, would improve lung function in patients with CF.

## PATIENTS AND METHODS

This study was undertaken in the outpatient CF clinics of the Royal Children's Hospital and the Alfred Hospital, Melbourne, Australia. Children, aged 7 years or older, and adults with a confirmed diagnosis of cystic fibrosis (sweat chloride above 60 mmol/L) who had clinically stable lung disease with an FEV<sub>1</sub> between 30% and 70% of predicted<sup>17</sup> were invited to participate in the study. Further inclusion criteria included the ability to perform reproducible pulmonary function tests; persistent cough with daily sputum production; regular performance of chest physiotherapy at home; a stable medication regimen for at least 14 days, and living within a reasonable distance from the clinic. All medications that were part of the regular treatment regimen were continued during the study period. Subjects who had a recent exacerbation of respiratory infection requiring hospital admission could enter the study no earlier than 4 weeks after discharge. Patients were excluded when there was an exacerbation of respiratory infection requiring admission to the hospital during the study period.

## Study Design

A prospective, open-label, placebo-controlled parallel group trial was performed. Although the subjects were not informed which study drug they were receiving, HS could be easily identified by subjects because of its salty taste; it was, therefore, assumed impossible to double blind the study. The technician making measurements of lung function was blind to the study solution that the subject was randomized to receive. Using random tables, eligible patients were randomized to receive either normal (0.9%) saline (IS) or hypertonic (6.0%) saline (HS). The study solution was inhaled twice daily for a period of 2 weeks prior to the patient's regular physiotherapy session. A portable ultrasonic nebulizer (Omron NE-U 07) with an output of 1 ml/min was used to inhale 10 ml of saline. Prior to the administration of saline, each patient had six puffs (600 µg) of salbutamol from a metered dose inhaler via a large volume spacer device (Volumatic). The first and last inhalations of study solution were administered in the clinic under a doctor's supervision and changes in lung function were measured.

After consent, all participating CF patients were seen in the outpatient clinic at baseline, on day 14 following 2 weeks of therapy, and on day 28 after 2 weeks off therapy to perform spirometry and to assess CF-related symptoms. On the first visit, the subject's medical history, details of chest physiotherapy, use of inhaled bronchodilators, and other medications were recorded and a physical examination was done. Subjects were excluded when the measured FEV<sub>1</sub> fell by more than 20% from baseline following the first treatment. Adverse side effects, intercurrent events, and use of medication were recorded at each subsequent visit. The study protocol was approved by the Human Ethics Committee of the Royal Children's and Alfred Hospital, and written informed consent was obtained from all subjects or their parents.

## Pulmonary Function Tests

Forced expiratory volume in 1 second (FEV<sub>1</sub>) and forced vital capacity (FVC) were measured with an electronic spirometer (Vitalograph Compact), using the standard methods for test performance recommended by the American Thoracic Society.<sup>18</sup> All patients were asked to refrain from taking bronchodilators for 6 hours before testing. At baseline and on day 14, pulmonary function tests were performed before and after inhalation of salbutamol and the study drug to identify those subjects who would develop significant bronchoconstriction. Further spirometry was done on day 28, 2 weeks after cessation of saline inhalation. The time of testing (a.m., midday, or p.m.) was kept constant for each individual. The lung function measurements used for analysis on day 0 and 14 were those made prior to administration of salbutamol and the study drug.

### Study Diary and Visual Analog Scores

As a subjective outcome measure, CF related symptoms were assessed on day 14 and day 28 in the form of perceived changes from baseline. Patients had to score dyspnea, fatigue, appetite, exercise tolerance, sleep, and general well-being on a 10 cm visual analog scale from -5 to +5 for each symptom over the preceding 2 weeks. Extreme worsening was indicated by a score of -5; 0 indicated no change, and +5 indicated maximum improvement. During the 2 week period of saline inhalation, subjects recorded twice daily their subjective feeling of a cleared chest on a diary card after an inhalation and physiotherapy session. Patients used the same visual analog scale (VAS), where -5 indicated chest tightness and less effective sputum clearance, 0 indicated no change, and +5 indicated the best improvement with a cleared chest.

### Statistics

The mean percentage change in FEV<sub>1</sub> and FVC from baseline were compared by using a two-sample t-test. Statistical tests were two sided, conducted at the 0.05 level. Assuming a within-group standard deviation of 16%, the necessary sample size (n) was 26 for each treatment group to show a 15% difference between groups in change from baseline FEV<sub>1</sub>, with  $\alpha = 0.05$ , and a power of 80%. The Mann-Whitney U test was used for analysis of the visual analogue scores. Results are presented as mean  $\pm$  SD.

## RESULTS

Fifty-eight patients, 43 children and adolescents from the Royal Children's Hospital and 15 adults from the Alfred Hospital, Melbourne, fulfilled the inclusion criteria and were randomized into the control and treatment groups. Six individuals were withdrawn during the study period due to noncompliance with clinic attendance (1 IS), exacerbation of respiratory infection requiring admission (2 IS, 1 HS), irritating cough during inhalation (1 HS), and increased hemoptysis (100 ml), which occurred 3 hours after the first treatment (1 HS). The baseline characteristics of the 52 subjects who completed the study are shown in Table 1. There were no significant differences between the two groups. The use of concomitant treatments, including topical or oral steroids and nebulized or oral antibiotics, was similar for the two treatment groups. No subject had a fall in FEV<sub>1</sub> >20% following inhalation of the study solution. One subject had a fall of 17%, and the remainder had either no change or a fall of less than 15%.

### Pulmonary Function Tests

After 2 weeks of administration of ultrasonically nebulized HS, pulmonary function was improved. The mean change in FEV<sub>1</sub> from baseline was an increase of 15.0%

( $\pm 16.0$ ) in the HS group, compared with 2.8% ( $\pm 13.1$ ) in the IS group ( $P = 0.004$ ; Fig. 1). On day 28, 2 weeks after discontinuation of saline inhalation, the mean FEV<sub>1</sub> returned to baseline (HS:  $+0.5 \pm 15.8\%$ , IS:  $-2.7 \pm 10.1\%$ ), with no difference between groups. The effect of HS on changes in FVC was smaller than the effect on FEV<sub>1</sub>. At the end of 14 days of HS therapy, there was a rise in FVC of  $8.0 \pm 13.4\%$  in the treatment group compared with a  $2.6 \pm 12.2\%$  change in the control group ( $P = \text{ns}$ ). On day 28 the FVC had returned to baseline in both groups (Fig. 2).

### Clinical Perceptions

There was a subjective improvement in the effectiveness of chest physiotherapy after HS inhalation. On the 10 cm VAS, all HS patients rated their subjective feeling of a cleared chest after every physiotherapy session during the 2 week period of treatment. For statistical analysis, we averaged the scores for the first and second week of treatment. The mean improvement in the VAS during the first week was  $2.05 \pm 1.3$  cm in patients on HS and  $1.17 \pm 1.25$  cm in patients on IS ( $P = 0.015$ ). For week 2 the mean rise was  $2.76 \pm 1.45$  cm on HS and  $1.75 \pm 1.6$  cm on IS ( $P = 0.02$ ; Fig. 3).

A similar VAS was used to assess changes in CF-related symptoms on days 14 and 28 compared to the preceding weeks. Administration of HS significantly improved the quality of sleep and exercise tolerance. Although there was an improvement, the differences between the two treatments did not reach statistical significance for the other disease-related symptoms and general well-being (Table 2). After discontinuation of treatment, however, symptom scores in both groups returned to baseline by day 28.

### Side Effects

On each clinic visit, adverse side effects and intercurrent events were recorded. Fourteen adverse events related to the respiratory tract were reported: six in the IS and eight in the HS group (Table 3). An increase in cough was noted by three patients in each group. In the HS group, one subject reported pharyngitis and one reported chest tightness. Intercurrent hemoptysis occurred in three patients on each treatment. In no case was the hemoptysis a new event, nor was it temporally related to the inhalation of saline. In one patient hemoptysis was severe enough to warrant withdrawal from the trial.

## DISCUSSION

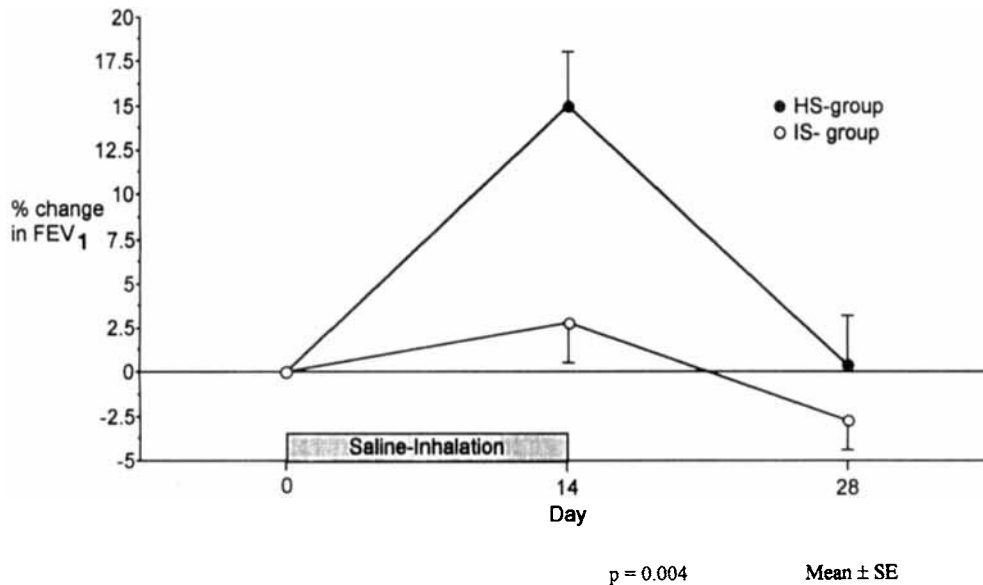
This study has shown a mean increase in FEV<sub>1</sub> of 15% after 2 weeks of inhaling HS in patients with CF with moderate to severe lung disease. A subjective improvement in the effectiveness of chest physiotherapy after use of HS and in CF-related symptoms was also noted.

**TABLE 1—Baseline Characteristics**

	Isotonic saline (n = 25)	Hypertonic saline (n = 27)
Mean age (yr); range	16.7 (7.6); range 8–36	16.1 (5.2); range 7–25
Sex (M/F)	13:12	18:9
Height (cm)	150 (19.6)	155 (17.7)
Weight (kg)	43 (16.2)	47 (14.5)
No. patients on		
Inhaled bronchodilators	21	21
Oral/inhaled steroids	11	9
FEV <sub>1</sub> (% predicted)	53.7 (7.8)	50.0 (9.7)
FVC (% predicted)	77.2 (9.8)	73.5 (15.9)

Results are expressed as mean ( $\pm$  SD).

There were no significant differences between the two groups.



**Fig. 1. Percent change in FEV<sub>1</sub> from baseline for each group (mean  $\pm$  SE).**

Hypertonic saline improves the effectiveness of cough, both as a protussive agent and by eliciting a change in mucus factors.<sup>19</sup> In a recent pilot study, we demonstrated sputum expectoration to be approximately doubled when ultrasonically nebulized HS was inhaled prior to the chest physiotherapy session compared with IS.<sup>13</sup>

Mucociliary clearance (MCC) is enhanced following inhalation of HS.<sup>15</sup> A single dose of aerosolised 7% HS significantly increases the amount of radioaerosol cleared from the lung when compared with amiloride and IS. Since both amiloride and HS induce cough in some patients, the effect of cough alone on MCC was also studied to eliminate a possible confounding effect. The difference between cough alone and HS remained significant.<sup>15</sup> This effect is in addition to the enhancement of MCC from cough alone. Furthermore, inhaled HS is likely to change the osmolality of the airway surface liquid.

The volume of airway surface liquid has been estimated

to be less than 1 ml,<sup>20</sup> so that deposition of a small amount of HS is likely to cause a significant change in osmolality, which draws water into the airways, leading to rehydration of airway secretions. Six percent HS has an osmolality of  $1862 \pm 5$  mOsm/kg. A recent study on the effect of hypertonic saline on the function of the pulmonary epithelial barrier showed that after instillation of hyperosmolar seawater (3% HS), there is a rapid influx of water from the plasma into the bronchoalveolar space.<sup>14</sup> The osmotic equilibration was completed within 3 minutes. Furthermore, there was no injury to the epithelial or endothelial barriers of the lung.

In this study, patients of the control group using IS had a small improvement in pulmonary function and tended to rate IS inhalation before physiotherapy as effective. Differences in changes of FEV<sub>1</sub> and some symptom scores were still significantly in favor of HS. Since IS inhalation has been shown to slightly enhance both nasal and pulmo-

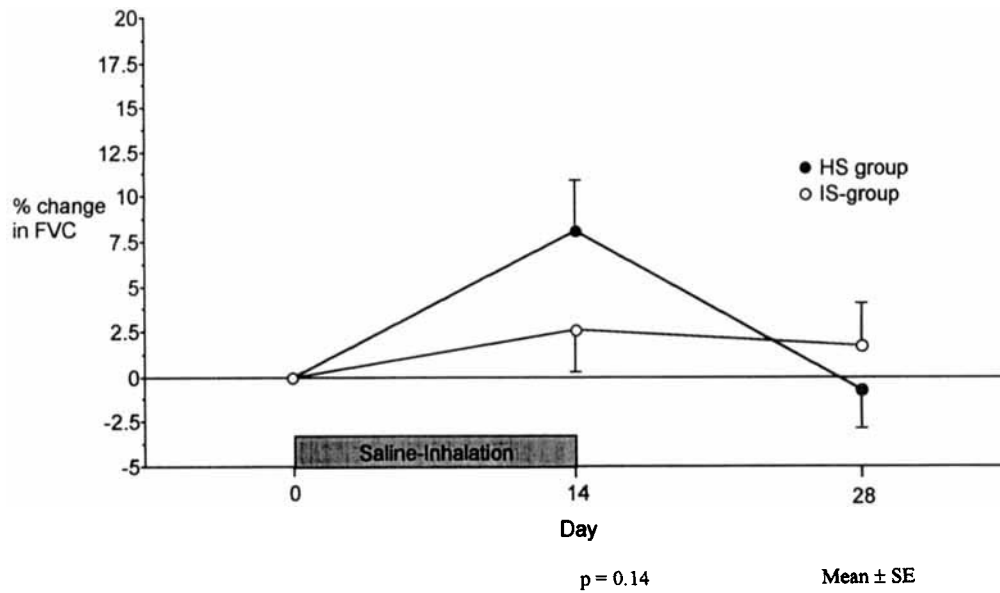


Fig. 2. Percent change in FVC from baseline for each group (mean  $\pm$  SE).

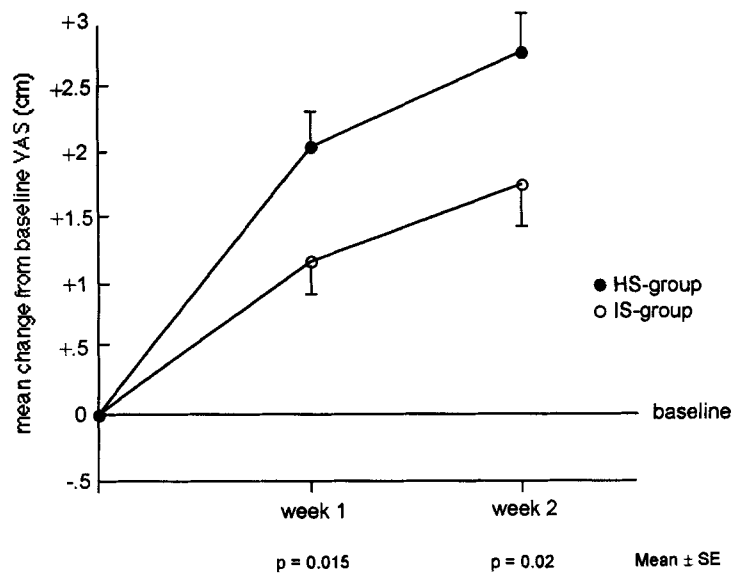


Fig. 3. The mean ( $\pm$ SE) change from baseline on the visual analog scale (VAS) for subjective feelings of a cleared chest following inhalation of HS or IS and a routine chest physiotherapy session.

nary MCC,<sup>15,21</sup> we consider the changes after IS inhalation to be most likely due to a therapeutic effect rather than a "placebo response" of the control group due to a better compliance with standard therapy during the study period.

Over the 2 week study period, HS was well tolerated. Hypertonic saline can induce cough and bronchoconstriction in some patients due to changes in the osmolality and ion concentrations of the airways.<sup>22,23</sup> This effect is confined to the period of inhalation and may enhance sputum expectoration. In the current study, a temporary

increase in cough was not considered to be a major inconvenience by most of the patients. One subject, however, had to be withdrawn because of intense cough induced by HS. Following this study, the same patient tried HS in a lower concentration (4.5%), which was well tolerated. This suggests that the tussive effect of saline is concentration dependent. Surprisingly, there was a subjective improvement in sleep quality in the HS group. This may be explained by less frequent disturbance caused by nocturnal coughing in patients using HS compared with IS.

**TABLE 2—Cystic Fibrosis—Related Symptoms on Day 14, After 2 Weeks of Treatment**

Symptom	Isotonic saline (n = 25)	Hypertonic saline (n = 27)	P value
Dyspnea	1.46 ± 1.8	2.07 ± 1.9	0.23
Fatigue	1.17 ± 1.6	1.85 ± 2.2	0.21
Appetite	0.46 ± 1.2	0.7 ± 1.6	0.53
Exercise tolerance	1.25 ± 2.0	2.33 ± 1.9	0.048
Quality of sleep	0.12 ± 1.5	1.3 ± 2.1	0.03
General well-being	1.58 ± 2.3	2.48 ± 1.7	0.11

Results are expressed as mean ± SD of changes from baseline on a 10 cm visual analog scale (cm).

**TABLE 3—Adverse Events**

	Isotonic saline (n = 25)	Hypertonic saline (n = 27)
Cough increase	3	3
Hemoptysis (intercurrent)	3	3
Tightness in chest	0	1
Pharyngitis	0	1
None (no. of patients)	19	21

Hypertonic saline is a nonspecific bronchial irritant that has been increasingly used for bronchial provocation tests in asthma patients.<sup>24,26</sup> Approximately 30% of patients with CF who have asthma-like symptoms will develop bronchoconstriction following inhalation of hyperosmolar saline.<sup>27</sup> Administration of 600 µg salbutamol prior to saline inhalation was effective in preventing significant bronchoconstriction in our study (i.e., >15% fall from baseline FEV<sub>1</sub>) in all but one CF patient who had a clinical history of asthma. Pulmonary function measurements 10 minutes after HS inhalation recorded a 17% fall from baseline FEV<sub>1</sub> in this individual, who subjectively experienced an increase in cough and some tightness in the chest during inhalation of HS. After 2 weeks of treatment, her lung function remained unchanged as compared with baseline. Therefore, we emphasize the need to assess the response of each patient with CF to inhaled HS before introducing it as a regular treatment.

Recombinant human DNase is now being used as an adjunct to standard therapy of CF and has been shown to improve lung function in both short and long-term studies.<sup>6,7</sup> In a phase II study of similar design as our protocol, twice daily administration of 2.5 mg recombinant human DNase over 10 days resulted in a 13.3% improvement in FEV<sub>1</sub> and a 7.2% increase in FVC. Two weeks following cessation of therapy, the lung function tests returned to baseline. These results are not different from those obtained using HS. In a major long-term study of 968 patients over a 24 week period, an improvement of 5.8% was maintained for FEV<sub>1</sub> and 3.8% for FVC.<sup>7</sup> The cost of this treatment is approximately US \$9,000 per annum. It is now important to determine whether HS

can provide a low cost alternative and achieve similar long-term results. In this study of short-term HS administration we were unable to examine its effect on reducing the incidence of respiratory infections. The topical antibacterial effect of concentrated saline solutions is well established.<sup>28,29</sup> Moreover, decreased sodium concentration, an electrolyte disturbance observed in CF airways, was recently found to inhibit human leukocytes in their ability to kill *Pseudomonas aeruginosa*.<sup>30</sup> These results suggest that therapy designed to increase the Na<sup>+</sup> concentration in CF airways may improve the leukocytes' ability to phagocytose bacteria, which combined with enhanced mucociliary clearance may reduce the incidence of respiratory tract infections.

*In conclusion*, this open label study has demonstrated that short-term administration of inhaled HS improves pulmonary function in children and young adults who have moderate to severe CF lung disease. In addition, there was subjective improvement in the effectiveness of chest physiotherapy after HS inhalation. These beneficial effects disappeared 2 weeks after discontinuation of treatment. It is now important to assess the efficacy of this therapy over a longer interval and to determine any additive benefits from combining recombinant human DNase with hypertonic saline.

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