

ORIGINAL ARTICLE

Mucus Clearance and Lung Function in Cystic Fibrosis with Hypertonic Saline

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ABSTRACT

BACKGROUND

Abnormal homeostasis of the volume of airway surface liquid in patients with cystic fibrosis is thought to produce defects in mucus clearance and airway defense. Through osmotic forces, hypertonic saline may increase the volume of airway surface liquid, restore mucus clearance, and improve lung function.

METHODS

A total of 24 patients with cystic fibrosis were randomly assigned to receive treatment with inhaled hypertonic saline (5 ml of 7 percent sodium chloride) four times daily with or without pretreatment with amiloride. Mucus clearance and lung function were measured during 14-day baseline and treatment periods.

RESULTS

Long-term inhalation of hypertonic saline without pretreatment with amiloride (i.e., with placebo pretreatment) resulted in a sustained (≥ 8 hours) increase in 1-hour rates of mucus clearance, as compared with those with amiloride pretreatment (14.0 ± 2.0 vs. 7.0 ± 1.5 percent, respectively; $P=0.02$) and increased 24-hour rates of mucus clearance over baseline. Furthermore, inhalation of hypertonic saline with placebo improved the forced expiratory volume in one second (FEV_1) between the baseline period and the treatment period (mean difference, 6.62 percent; 95 percent confidence interval, 1.6 to 11.7; $P=0.02$), whereas hypertonic saline with amiloride did not improve FEV_1 (mean difference, 2.9 percent; 95 percent confidence interval, -2.2 to 8.0; $P=0.23$). Forced vital capacity (FVC), the forced expiratory flow between 25 and 75 percent of FVC (FEF_{25-75}), and respiratory symptoms also significantly improved in patients treated with hypertonic saline and placebo, whereas the residual volume as a proportion of total lung capacity ($RV:TLC$) did not change in either group. A comparison of the changes in lung function in the two groups showed no significant difference. In vitro data suggested that sustained hydration of airway surfaces was responsible for the sustained improvement in mucus clearance, whereas inhibition of osmotically driven water transport by amiloride accounted for the observed loss of clinical benefit.

CONCLUSIONS

In patients with cystic fibrosis, inhalation of hypertonic saline produced a sustained acceleration of mucus clearance and improved lung function. This treatment may protect the lung from insults that reduce mucus clearance and produce lung disease.

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MUCUS CLEARANCE DEFENDS THE LUNG against inhaled bacteria. The efficiency of mucus clearance depends on an adequate volume of airway surface liquid (i.e., hydration).¹ One hypothesis for the pathogenesis of lung disease in patients with cystic fibrosis is that a lack of regulation of sodium absorption and chloride secretion causes depletion of airway surface liquid, slows mucus clearance, and promotes the formation of adherent mucus plaques on airway surfaces. Mucus plaques and plugs obstruct airways and provide the nidus for infection.^{2,3}

On the basis of this hypothesis, therapies that increase the volume of airway surface liquid, and hence mucus clearance, should improve lung disease in patients with cystic fibrosis. Inhaled hypertonic saline has been shown to produce short-term stimulation of mucus clearance^{4,5} and, in separate studies, to improve lung function.^{6,7} In vitro studies with normal human airway epithelia demonstrated that hypertonic saline increases the volume of airway surface liquid, but the effects were transient and, therefore, predicted to be of limited therapeutic benefit.⁸ These in vitro studies, however, demonstrated that slowing the absorption of sodium with amiloride, a sodium-channel blocker, significantly extended the duration of the increase in the volume of airway surface liquid. We tested the hypothesis that pretreatment with amiloride would extend the duration of hypertonic saline-induced acceleration of mucus clearance and enhance improvement in lung function in patients with cystic fibrosis.

METHODS

STUDY DESIGN

The study protocol was approved by the University of North Carolina Committee on the Protection of Rights of Human Subjects, and written informed consent was obtained. Patients were enrolled between January 2001 and February 2004. Inclusion criteria included an established diagnosis of cystic fibrosis, an age of at least 14 years, and a forced expiratory volume in one second (FEV₁) of 50 percent or more of the predicted value after bronchodilation. Exclusion criteria included unstable lung disease (as evidenced by the administration of intravenous antibiotics within four weeks before screening, a change of medical regimen within two weeks before screening, or

an FEV₁ \geq 15 percent below the best clinical value during the previous six months), evidence of reactive airways ($>$ 15 percent response to bronchodilation or clinical diagnosis of asthma), and the use of hypertonic saline within two weeks before screening. (For details, see the Supplementary Appendix, which is available with the full text of this article at www.nejm.org.)

After screening, patients with cystic fibrosis entered a 14-day baseline observation period (Fig. 1), after which they were randomly assigned in a 1:1 distribution to pretreatment with either amiloride (at a dose of 1 mg per milliliter in 4.5 ml of 0.12 percent sodium chloride [Sifavitor]) or taste-masked placebo (at a dose of 0.25 mg of quinine sulfate per milliliter in 4.5 ml of 0.12 percent sodium chloride [DSM Minera]) before the administration of 7 percent sodium chloride (at a dose of 5 ml). Aerosols were delivered four times daily with the use of a Pari LC Star nebulizer and Pari Proneb Ultra compressor (Pari). Albuterol (at a dose of 180 μ g) was administered with a metered-dose inhaler with a spacer 30 to 60 minutes before the administration of study medications.

STUDY OUTCOMES

The prespecified outcomes of the primary study were the percentage changes in FEV₁ and forced vital capacity (FVC) during the treatment period. Secondary outcomes were the rates of mucus clearance, the forced expiratory flow between 25 and 75 percent of FVC (FEF₂₅₋₇₅), the ratio of residual volume to total lung capacity (RV:TLC), and quality of life. The latter was measured with the Cystic Fibrosis Questionnaire for teens and adults, in which domain scores range from 0 to 100, with higher scores reflecting better perceived health status.⁹

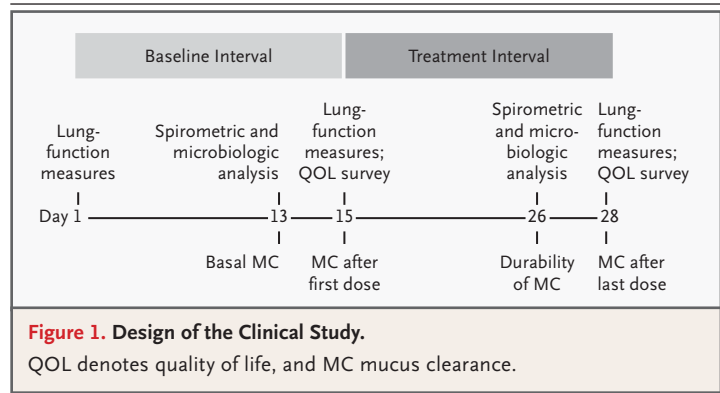
Whole-lung mucus clearance was measured, as previously described,^{10,11} four times during the study: during the baseline interval (basal mucus clearance on day 13), immediately after the first dose of study medications (mucus clearance after the first dose on day 15), during long-term use of study medication but eight or more hours after administration of the preceding dose to assess sustained effects (durability of mucus clearance on day 26), and immediately after the administration of study medications at the end of the treatment interval (mucus clearance after the last dose on day 28). The 1-hour rate of mucus clear-

ance, calculated from the average of measurements at 10-minute intervals, served as the primary index of mucociliary clearance. Patients returned 24 hours after the administration of radioisotopes to measure the cumulative mucus clearance.

For comparison, data describing mucus clearance were collected from 15 healthy, nonsmoking adults (mean [\pm SD] age, 25.6 \pm 3.9 years; FEV₁, 103 \pm 9.8 percent of the predicted value) with the use of identical techniques, except that healthy subjects received pretreatment with normal saline (2.5 ml over a period of 30 minutes, delivered by de Vilbiss 646 nebulizer), rather than with albuterol. Neither the administration of albuterol in patients with cystic fibrosis nor saline by nebulizer in controls was expected to affect mucus clearance.¹²

Spirometric and plethysmographic measurements of lung volumes were performed according to American Thoracic Society standards,¹³ and the percentage changes during baseline and treatment periods were calculated. Spirometric measurement was also performed two hours after the administration of study medications on day 15 and day 28 and compared with values before administration. Quantitative sputum cultures and serum chemical profiles were performed before and at completion of the treatment period.

In vitro experiments were performed using bronchial epithelia obtained and cultured as previously described.² Airway surface liquid was labeled with 2 mg of Texas red dextran per milliliter in 20 μ l of phosphate-buffered saline, and the volume of airway surface liquid was monitored by axial confocal microscopy.² Sodium chloride (at a dose of 0.8 mg) with or without amiloride (final concentration, 400 μ M) was added (in perfluorocarbon) to the mucosal surface of epithelial cultures from patients with cystic fibrosis and from controls. The volume of airway surface liquid was serially measured before and after the addition of sodium chloride. The contribution of chloride transport by the cystic fibrosis transmembrane conductance regulator (CFTR) to the change in the volume of airway surface liquid in normal epithelia after the administration of sodium chloride was assessed by pretreating cultures with a CFTR inhibitor (CFTR_{inh}-172 at a concentration of 5 μ M)¹⁴ or by substituting the impermeant anion gluconate for chloride (3.0 mg of sodium gluconate with 20 mM of calcium chloride to maintain calcium activity¹⁵).



The effect of amiloride on transepithelial water transport was measured by the addition of mannitol (at a concentration of 300 mM in 100 μ l of phosphate-buffered saline) with or without amiloride (at a concentration of 400 μ M) to the apical surface of airway epithelial cultures from patients with cystic fibrosis to generate an osmotic driving force. Water flow was quantitated by measuring the resulting change in serosal bath fluorescence.¹⁶ (Additional in vitro methods are described in the Supplementary Appendix.)

STATISTICAL ANALYSIS

Sample size was based on the ability to detect a treatment effect equal to 1 SD of the FEV₁. A sample size of 16 patients per group provided 80 percent power to detect this treatment effect with the use of a two-sided test ($\alpha=0.05$). We anticipated a reduced variance and an increase in power because two FEV₁ measures were averaged to produce each end of interval values.

Univariate data analyses were performed with the use of paired or unpaired two-sided t-tests, as appropriate, with a P value of 0.05 or less accepted as indicating significance. Patients who had undergone randomization and who had received at least one dose of study medication and contributed any data during the treatment interval were included in a modified intention-to-treat analysis.

For studies of mucus clearance, the ratio of particle deposition in central regions of the lung, as compared with peripheral regions (C:P ratio), was calculated for each mucus-clearance scan. The 1-hour and 24-hour mucus-clearance rates were calculated and compared with use of paired and unpaired t-tests, as appropriate. The effect of study treatments on mucus clearance was also

tested with a mixed-model analysis of three components of variance that included the study visit, group assignment, and the C:P ratio as explanatory variables. Differences that are reported as significant were supported by both statistical methods.

RESULTS

DEMOGRAPHIC CHARACTERISTICS

Of the 29 patients who underwent screening, 27 met eligibility criteria and were enrolled. Three subjects were withdrawn before randomization because of pulmonary exacerbations (two patients) or hyperkalemia (one patient). At baseline, the treatment groups were well matched (Table 1). Three patients who underwent randomization did not contribute data to treatment-interval end points owing to adverse events and were not included in the modified intention-to-treat analysis. These events included a pulmonary exacerbation (in the group receiving hypertonic saline with placebo); probable abdominal sepsis in a patient with immunosuppression after liver transplantation (in the group receiving hypertonic saline with

amiloride); and an 18.6 percent drop in the FEV₁ two hours after the administration of the first dose of study drugs (in the group receiving hypertonic saline with amiloride), which was a predefined criterion for exclusion. Other adverse events were rare or mild in severity (as listed in the Supplementary Appendix).

BASAL MUCUS CLEARANCE

The mean (\pm SE) one-hour mucus-clearance rate, which is dominated by large-airway clearance, did not differ significantly between patients with cystic fibrosis (9.3 ± 1.1 percent) and controls (10.0 ± 1.7 percent) (mean difference, 0.6; 95 percent confidence interval, -3.3 to 4.7 ; $P=0.73$) (Fig. 2A and 2B). However, mucus clearance at 24 hours was reduced in patients with cystic fibrosis, as compared with controls (40.7 ± 2.5 vs. 53.5 ± 2.8 percent, $P<0.001$). This difference in the 24-hour measure did not reflect altered tracer deposition, as the C:P mean (\pm SE) ratios of particle deposition did not differ significantly between patients with cystic fibrosis (1.48 ± 0.05) and controls (1.57 ± 0.06) (mean difference, 0.09; 95 percent confidence interval, -0.07 to 0.25 ; $P=0.26$).

Table 1. Characteristics of the Patients at Baseline.*

	Hypertonic Saline with Amiloride (N=12)	Hypertonic Saline with Placebo (N=12)
Male sex — no. of patients	6	4
Age — yr	25 \pm 7	27 \pm 10
Δ F508 homozygosity — no. of patients (%)	6 (50)	7 (58)
FVC — % of predicted value	90 \pm 15	90 \pm 13
FEV ₁ — % of predicted value	78 \pm 18	78 \pm 19
FEF ₂₅₋₇₅ — % of predicted value	59 \pm 36	62 \pm 41
Concomitant therapies — no. of patients (%)		
Recombinant human deoxyribonuclease	4 (33)	4 (33)
Long-term macrolides	3 (25)	3 (25)
Inhaled steroids	6 (50)	6 (50)
Previous use of hypertonic saline	2 (17)	2 (17)
Coexisting illness — no. of patients (%)		
Liver disease	2 (17) [†]	2 (17)
Diabetes	2 (17)	2 (17)
Exocrine pancreatic insufficiency	12 (100)	10 (83)

* Plus-minus values are means \pm SD. FVC denotes forced vital capacity, FEV₁ forced expiratory volume in one second, and FEF₂₅₋₇₅ forced expiratory flow between 25 and 75 percent of FVC.

[†] Patients underwent liver transplantation.

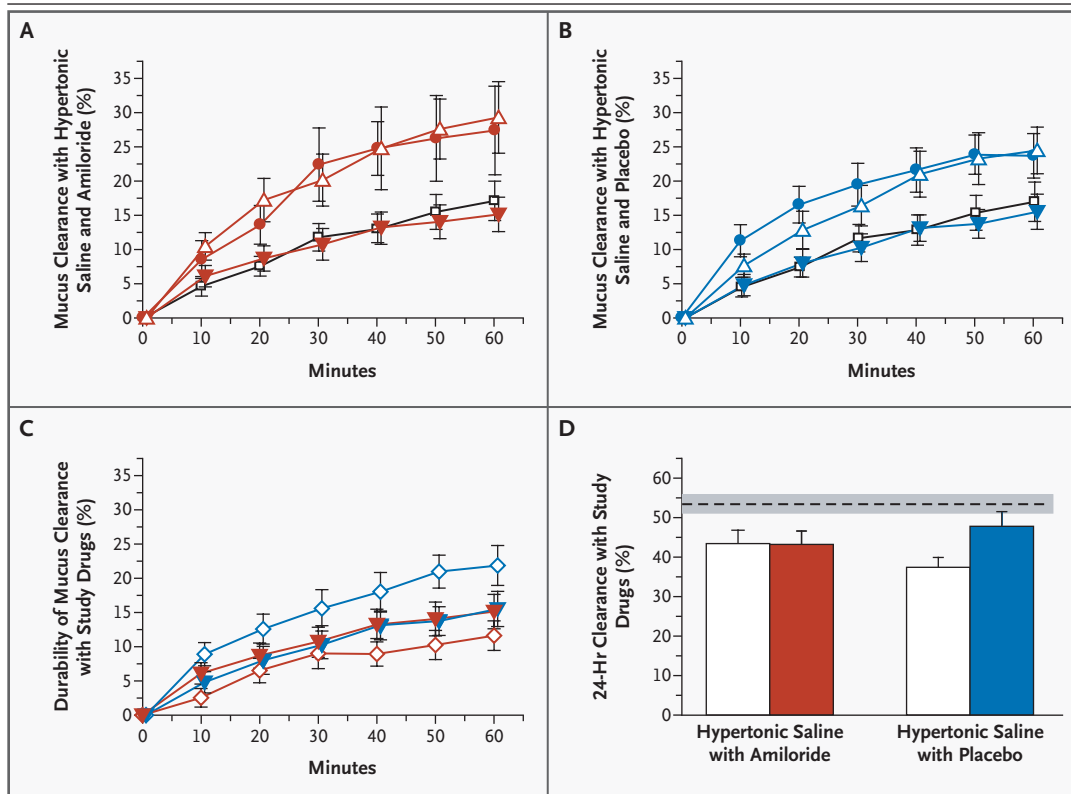


Figure 2. Effect of Study Medications on Mucus Clearance.

Panel A shows the percentage of particles cleared from the whole lung compartment during the first hour after deposition in patients with cystic fibrosis who received hypertonic saline after pretreatment with amiloride. Baseline mucus clearance (solid inverted triangles), mucus clearance immediately after the first dose of study medications (open triangles), and mucus clearance immediately after study medications at the end of the treatment interval (solid circles) in patients with cystic fibrosis are shown in red. Baseline mucus clearance in controls (open black squares) is provided for comparison ($P \leq 0.05$ for the comparison of basal mucus clearance and clearance after the first dose of hypertonic saline and amiloride). Panel B shows the percentage of particles cleared from the whole lung compartment during the first hour after deposition in patients with cystic fibrosis who received hypertonic saline after placebo. Baseline mucus clearance (solid inverted triangles), mucus clearance immediately after the first dose of study medications (open triangles), and mucus clearance immediately after study medications at the end of the treatment interval (solid circles) in patients with cystic fibrosis are shown in blue. Baseline mucus clearance in controls (open black squares) is provided for comparison ($P \leq 0.05$ for the comparisons between basal mucus clearance and clearance after both the first and last doses of study medications). Panel C shows the sustained effects of study medications on mucus clearance, which was measured ≥ 8 hours after administration on day 26 (open diamonds), as compared with baseline (solid triangles) in patients with cystic fibrosis treated with hypertonic saline and amiloride (red) or hypertonic saline and placebo (blue) ($P \leq 0.05$ for both the comparison of the durable-clearance measurements at the end of the treatment interval between the treatment and control groups and the comparison between basal and durable-clearance measures in the group that received hypertonic saline after placebo). Panel D shows 24-hour clearance (mean \pm SE) measured before study medications (white bars) on study day 26 after long-term treatment with hypertonic saline and amiloride (red) or hypertonic saline and placebo (blue) ($P \leq 0.05$ for the comparison between baseline and post-treatment 24-hour clearance values in the group that received hypertonic saline with placebo). The 24-hour clearance in controls is shown for comparison, with the mean (\pm SE) plotted as a dotted line and shaded area.

EFFECT OF HYPERTONIC SALINE WITH OR WITHOUT AMILORIDE

Baseline 1-hour mucus-clearance rates and cumulative mucus clearance at 24 hours (on day 13) did not differ in the two treatment groups (Fig. 2).

The one-hour mucus-clearance rate that was measured immediately after the first dose of study medications (on day 15) was significantly increased relative to baseline in both groups ($P = 0.01$ and $P = 0.05$ in groups receiving hypertonic saline with

amiloride and with placebo, respectively) (Fig. 2A and 2B). The one-hour mucus-clearance rate that was measured immediately after study medications on the final day of treatment (on day 28) was similarly increased relative to basal mucus clearance, suggesting no diminution in the peak effects on mucus clearance over this two-week period (Fig. 2A and 2B).

There were two important differences in the effects of treatment regimen on mucus clearance during long-term administration. First, the assessment of the durability of mucus clearance revealed a significantly faster one-hour mucus-clearance rate in the group receiving hypertonic saline with placebo than in the group receiving hypertonic saline with amiloride (14.0 ± 2.0 vs. 7.0 ± 1.5 percent, $P=0.02$) (Fig. 2C). This difference reflected the fact that only hypertonic saline with placebo produced a sustained increase (≥ 8 hours) in the durability measurement of one-hour mucus clearance (14.0 ± 2.0 percent) versus that for the basal measurement of mucus clearance (9.3 ± 1.6 percent; $P=0.04$) (Fig. 2C). Second, although the cumulative 24-hour mucus-clearance rate was not significantly higher in the group that received hypertonic saline with placebo at the durability-of-mucus-clearance scan (mean difference, 4.6 percent; 95 percent confidence interval, -5.9 to 15.1 ; $P=0.36$), long-term administration of hypertonic saline with placebo increased the 24-hour rate as compared with pre-treatment values ($P=0.04$), whereas long-term administration of hypertonic saline with amiloride did not increase the rate ($P=0.49$) (Fig. 2D).

LUNG FUNCTION

Similar small changes in lung function were observed during the baseline interval in both treatment groups (Fig. 3). The absolute percentage changes in lung function during the treatment interval favored the group that received hypertonic saline with placebo but were not significantly different (mean FEV₁ difference, 4.7 percent; 95 percent confidence interval, -1.3 to 10.6 ; $P=0.12$; mean FVC difference, 1.8 percent; 95 percent confidence interval, -1.9 to 5.5 ; $P=0.23$; mean FEF₂₅₋₇₅ difference, 13.1 percent; 95 percent confidence interval, -1.4 to 27.7 ; $P=0.07$). The corresponding absolute increases in FEV₁ resulting from treatment were 147 ± 39 ml for the group receiving hypertonic saline with placebo and 34 ± 66 ml for the group receiving hypertonic

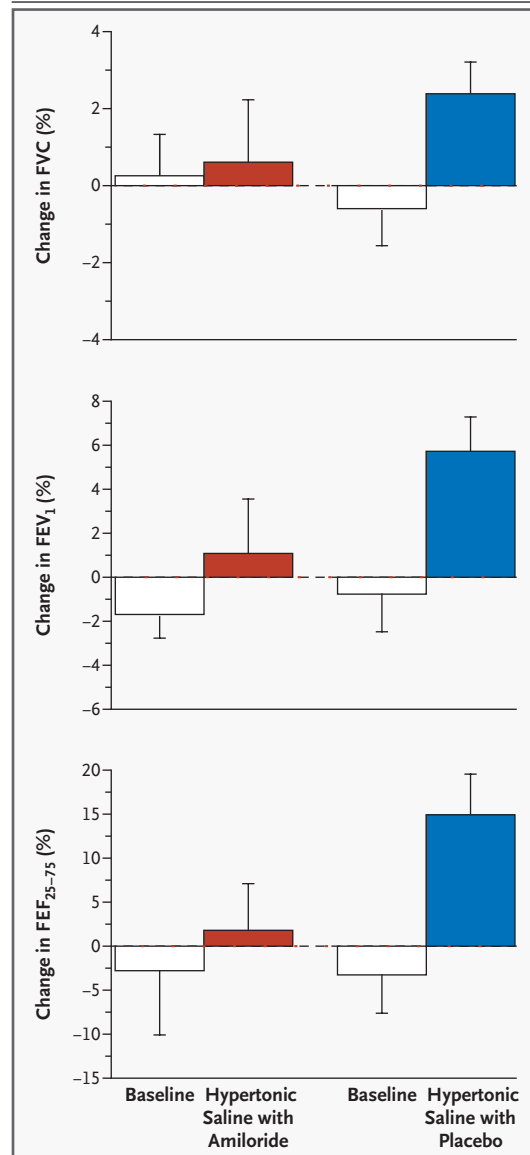


Figure 3. Effect of Study Medications on Lung Function.

The mean (\pm SE) percentage change in FVC, the FEV₁, and the FEF₂₅₋₇₅ during the baseline (white bars) and treatment intervals are shown for each treatment group, indicating the results of administration of hypertonic saline with amiloride (red) and with placebo (blue) ($P<0.05$ for comparisons between baseline and post-treatment measures in the group receiving hypertonic saline after placebo). T bars indicate standard errors.

saline with amiloride (mean difference, 113 ml; 95 percent confidence interval, -44 to 271). However, comparisons of changes in lung function during the treatment and baseline intervals revealed significant improvements in FVC ($P=0.05$), FEV₁ ($P=0.02$), and FEF₂₅₋₇₅ ($P=0.02$) in the group

that received hypertonic saline with placebo but no differences in the group that received hypertonic saline with amiloride ($P=0.83$, $P=0.23$, and $P=0.55$, respectively) (Fig. 3). No significant differences were observed in RV:TLC ratios.

QUALITY OF LIFE

The mean (\pm SE) respiratory symptom score as measured after the treatment interval was significantly better in the group that received hypertonic saline with placebo than in the group that received hypertonic saline with amiloride (82.3 ± 3.1 vs. 70.0 ± 3.1 , $P=0.01$). Significant improvement in respiratory symptoms but a higher perceived workload or burden related to medical treatment was also observed in a comparison of scores at baseline and during treatment in the group that received placebo but not in the group that received amiloride (complete questionnaire results are available in the Supplementary Appendix).

SAFETY AND ADVERSE EVENTS

No treatment-related serious adverse events occurred. Mean (\pm SE) absolute changes in FEV₁ two hours after the administration of study medication on day 15 (0.85 ± 3.79 percent in the placebo group and -2.47 ± 6.66 percent in the amiloride group) and on day 28 (-0.55 ± 4.46 percent in the placebo group and -1.39 ± 3.85 percent in the amiloride group) were small and not significantly different between groups. Serum chemical analyses, hematologic values, and quantitative sputum cultures (for total bacteria and pseudomonas) did not change with either treatment (see the Supplementary Appendix).

IN VITRO RESPONSE TO HYPERTONIC SALINE

The addition of sodium chloride to the mucosal surface of airway epithelial cultures, simulating deposition of 7 percent sodium chloride,¹⁰ produced a much larger and more sustained increase in the volume of airway surface liquid in airway cultures from patients with cystic fibrosis than in cultures from controls (Fig. 4A and 4B). We tested the hypothesis that the sustained response in cultures from patients with cystic fibrosis reflected the absence of the CFTR chloride channel as a route for transcellular salt absorption. Control cultures had a significantly increased response in the volume of airway surface liquid to hypertonic challenge when pretreated with a CFTR chloride-channel blocker (CFTR_{inh}-172) or with

replacement of chloride in the apical hypertonic solution with an impermeant anion (Fig. 4B). These data indicate that chloride transport through CFTR is a key determinant of the response in the volume of airway surface liquid to the administration of hypertonic saline.

INHIBITION OF TRANSEPIThELIAL WATER TRANSPORT BY AMILORIDE

The capacity of hypertonic saline to produce large and prolonged increases in the volume of airway surface liquid in airway epithelial cultures of patients with cystic fibrosis was markedly reduced by pretreatment with amiloride (Fig. 4A and 4C). The slower increase in serosal fluorescence in response to an apical hyperosmolar mannitol solution suggested that the reduced response in the volume of airway surface liquid to hypertonic saline after amiloride pretreatment reflected a block of transepithelial water flow (Fig. 4D). This effect was not specific to epithelia from patients with cystic fibrosis, since amiloride also slowed osmotically driven water flow in control epithelia; the rate of increase in fluorescence was reduced by 74.6 ± 4.0 percent in controls, as compared with 89.8 ± 2.3 percent in patients with cystic fibrosis ($P=0.01$).

The action of amiloride to block the water permeability of airways was further characterized. First, the 50 percent inhibitory concentration (IC₅₀) for amiloride inhibition of water permeability was $6\ \mu\text{M}$, a value that is less potent by a factor of 10 than that for inhibition of the epithelial sodium channel. Second, a cellular site of action was indicated by experiments in which a blocker of cellular aquaporins, mercuric chloride, mimicked the blockade by amiloride of hypertonic saline-induced expansion of airway surface liquid; amiloride also inhibited hypertonic saline-induced cell shrinkage. Finally, the amiloride blockade was pharmacologically specific, since an analogue that more potently inhibits the epithelial sodium channel (benzamil) was active, whereas an analogue more specific for the sodium-hydrogen exchanger (dimethylamiloride) was not (additional in vitro data are available in the Supplementary Appendix).

DISCUSSION

Treatment of patients who have cystic fibrosis with inhaled hypertonic saline after placebo re-

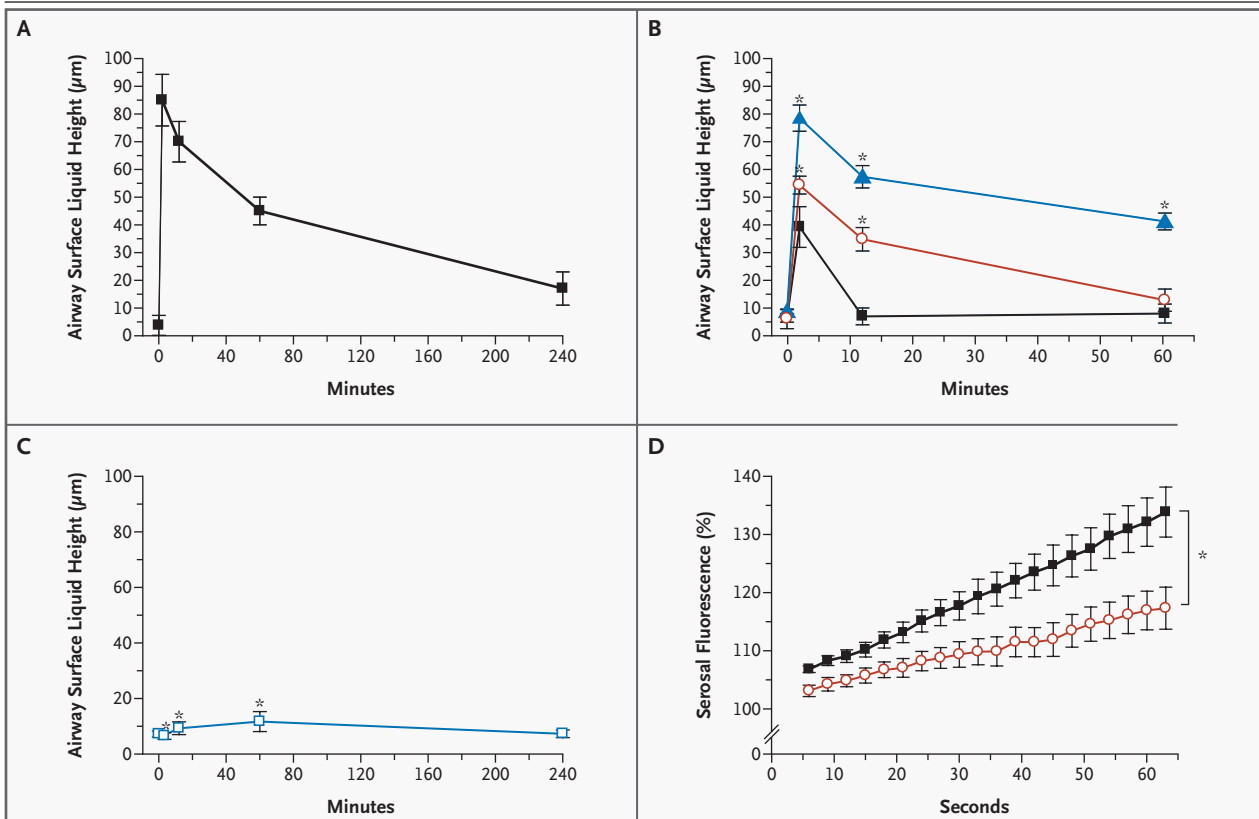


Figure 4. Effects of Hypertonic Saline with or without Amiloride on the Amount of Airway Surface Liquid in Epithelia Cultured from Patients with Cystic Fibrosis and Controls.

Panel A shows the amount of airway surface liquid (measured linearly in micrometers by confocal microscopy) before and after the addition of 0.8 mg of sodium chloride to epithelia from patients with cystic fibrosis. Panel B shows the amount of airway surface liquid in the epithelia from controls before and after 0.8 mg of sodium chloride (solid black squares). The effect of pretreatment with CFTR inhibitor 172 (open red circles) or chloride substitution with gluconate (solid blue triangles) is also depicted. Asterisks denote a significant difference in time points between treatment groups and controls ($P < 0.05$). Panel C shows the amount of airway surface liquid before and after the addition of 0.8 mg of sodium chloride to the apical surface of airway epithelial cultures from patients with cystic fibrosis pretreated with amiloride. Asterisks denote a significant difference in time points as compared with those in Panel A ($P < 0.05$). Panel D shows the change in transepithelial water transport (as measured by serosal bath fluorescence) in response to the addition of mannitol to the apical surface of airway epithelial cultures from patients with cystic fibrosis who either had pretreatment with amiloride (open red circles) or had no pretreatment (solid black squares). The asterisk denotes a significant difference in the slope of lines fitted through data points ($P < 0.05$). The I bars denote means \pm SE.

sulted in a sustained increase in the one-hour mucus-clearance rate and was associated with improvements in lung function and respiratory symptoms over baseline values. In contrast, patients who were treated with hypertonic saline after amiloride had no sustained increase in mucus clearance and no improvement in lung function or respiratory symptoms. The relative effectiveness of hypertonic saline treatment alone, as compared with the administration of hypertonic saline after pretreatment with amiloride, was contrary to our hypothesis. These findings raised two questions: Why was the effect of hypertonic

saline on mucus clearance so prolonged in patients with cystic fibrosis as compared with controls?²⁸ And why did amiloride blunt the ability of hypertonic saline to produce sustained increases in mucus clearance? We investigated these questions in airway cultures because a tight linkage between the volume of airway surface liquid and mucus transport has been demonstrated in this model system.¹

The administration of hypertonic saline in vitro produced a larger and more sustained increase in the volume of airway surface liquid in cultures from patients with cystic fibrosis than in

cultures from controls (Fig. 4A and 4B). The role of CFTR in the dissipation of the high luminal salt concentration that is generated by hypertonic saline deposition was revealed with pharmacologic blockade of CFTR and chloride-replacement studies (Fig. 4B). The resulting prolongation of an osmotic driving force would draw more water onto the airway surface in patients with cystic fibrosis, increase the volume of airway surface liquid, and produce a sustained increase in mucus clearance.

The effect of amiloride in blunting clinical improvements with hypertonic saline might also be explained by effects on the volume of airway surface liquid. In vitro experiments revealed markedly reduced expansion of airway surface liquid in response to the administration of hypertonic saline after pretreatment with amiloride (Fig. 4C) owing to the blockade of apical membrane water permeability (Fig. 4D and the Supplementary Appendix). As a result, the administration of hypertonic saline with amiloride may increase the tonicity of airway surface liquid, which through electrostatic effects on mucins may account for the acute increase in mucus clearance.^{17,18} It also may limit the expansion in the volume of airway surface liquid, preventing the sustained increase in mucus clearance and improvement in lung function observed with hypertonic saline alone.

In another article in this issue of the *Journal*, Elkins et al.¹⁹ also observed a modest improvement in lung function with inhaled hypertonic saline but observed a striking reduction in pulmonary exacerbations. We propose that hypertonic saline produced the sustained airway surface hydration required to clear retained mucus, but its efficacy was limited by its failure to reach many obstructed airways, as evidenced by the moderate resulting increase in FEV₁.¹⁹ In contrast, effective delivery of hypertonic saline to relatively nonob-

structed airways produced supranormal 1-hour mucus-clearance rates and improved cumulative 24-hour mucus-clearance rates. We speculate that the durable increase in mucus clearance protected relatively nonobstructed lung regions from exogenous insults (e.g., viral infections^{20,21}) that slow mucus clearance and hence promote intrapulmonary spread of bacterial infection or the development of new mucus obstruction, thus accounting for the large reduction in the exacerbation rate observed by Elkins et al.

In summary, inhalation of hypertonic saline four times daily provides a modest improvement in lung function and respiratory symptoms without substantial adverse events. On the basis of studies of airway epithelia in vitro, it appears likely that the mechanism of action of hypertonic saline is to provide long-term hydration of airway surfaces and promote a sustained increase in mucus clearance. This study points to a vital therapeutic role for the restoration of hydration to airway surfaces in patients with cystic fibrosis and suggests that measures of sustained mucus clearance may serve as a useful surrogate outcome for future drug development.

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Drs. Knowles and Boucher report having received consulting fees from Inspire Pharmaceuticals and having equity interests in Inspire Pharmaceuticals and Parion Sciences. Dr. Boucher reports having an equity interest in Respirics. Dr. Boucher was named coinventor on a patent for dry-powder amiloride, which was subsequently licensed to Parion Sciences. He also holds multiple other patents on compounds designed to treat lung diseases. No other potential conflict of interest relevant to this article was reported.

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