Short paper

Effect of increasing doses of hypertonic saline on mucociliary clearance in patients with cystic fibrosis

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Abstract

***Background* – Patients with cystic fibrosis are known to have decreased mucociliary clearance. It has previously been shown that inhalation of a 7.0% solution of hypertonic saline significantly improved mucociliary clearance in a group of adult patients with cystic fibrosis. The aim of this study was to measure the response to increasing concentrations of inhaled hypertonic saline.**

***Methods* – Ten patients (seven men) of mean (SE) age 22 (4) years and mean forced expiratory volume in one second (FEV_1) 52.0 (6.7)% predicted completed the study. Mucociliary clearance was measured using a radioaerosol technique for 90 minutes after the interventions which comprised 0.9% NaCl + voluntary cough (control), 3.0% NaCl, 7.0% NaCl, and 12% NaCl.**

***Results* – There was a significant increase in the amount of activity cleared from the right lung with all concentrations of hypertonic saline (HS) compared with control. The amount cleared at 90 minutes on the control day was 12.7% (95% confidence interval (CI) 9.8 to 17.2) compared with 19.7% (95% CI 13.6 to 29.5) for 3% HS, 23.8% (95% CI 15.9 to 36.7) for 7% HS and 26.0% (95% CI 19.8 to 35.9) for 12% HS. The improvement in mucociliary clearance was not solely due to coughing as the number of coughs recorded on the control day exceeded that recorded on any other day. The hypertonic saline did not induce a clinically significant change in FEV_1.**

***Conclusions* – Within the range of concentrations examined in this study, the effect of hypertonic saline appears to be dose dependent. Inhalation of hypertonic saline remains a potentially useful treatment for patients with cystic fibrosis.**

(*Thorax* 1997;**52**:900–903)

Keywords: cystic fibrosis, hypertonic saline, mucociliary clearance.

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Received 20 January 1997
Returned to authors 13 March 1997
Revised version received 22 April 1997
Accepted for publication 3 June 1997

Patients with cystic fibrosis are known to have impaired mucociliary clearance.[1] This is believed to play a pivotal role in the vicious cycle of airway obstruction, infection, and ongoing lung damage that is characteristic of this disease. A major goal of therapy in cystic fibrosis is the effective removal of thick tenacious secretions.

Using a radioaerosol technique we have previously shown that mucociliary clearance was markedly increased in a group of patients with cystic fibrosis following a single inhalation of a 7% solution of hypertonic saline.[2] In this study both isotonic saline and matched voluntary cough were used as controls. Clearance was measured for 60 minutes after each of the interventions. At that time the amount cleared from the whole right lung was 29.4 (6.4)% for hypertonic saline and 17.5 (4.5)% and 19.5 (4.7)% for the isotonic saline and matched voluntary cough, respectively ($p<0.01$). As the mean number of coughs recorded on the matched voluntary cough day (132 (20)) exceeded the number recorded on the hypertonic saline day (87 (21)), cough alone was not thought to be responsible for the observed increase in mucociliary clearance.

The aim of the present study was to perform a dose-response trial to determine the concentration of nebulised hypertonic saline that maximises the beneficial effects on mucociliary clearance whilst monitoring for any potential undesirable effects.

Methods

PATIENTS

The study group consisted of 10 adult patients with cystic fibrosis. The mean age was 22.1 (3.8) years (range 19–28) with a mean forced expiratory volume in one second (FEV_1) of 52.0 (6.7)% predicted (range 31–84). All patients were studied in a stable clinical condition and baseline medications were not altered throughout the study period. All the patients were chronically colonised with *Pseudomonas aeruginosa*. In addition, five of the patients had *Staphylococcus aureus*.

STUDY PROTOCOL

After inhalation of a standardised dose of bronchodilator (5 mg albuterol in 2.5 ml saline) via an ultrasonic nebuliser the patients inhaled

Table 1 Mean (SE) values for baseline mucociliary clearance (MCC) and percentage clearance immediately after intervention (%C30) and an hour later (%C90) and mucociliary clearance rate (AUC)

	Baseline MCC	*%C30*	*%C90*	*AUC*
Control	9.6 (4.4)	8.7 (1.7)	12.7 (1.4)	5895 (23)
3% HS	9.3 (9.8)	13.5 (2.6)*	19.7 (3.1)*	5775 (45)
7% HS	9.7 (8.0)	15.8 (3.0)*	23.8 (4.0)*	5675 (22)*
12% HS	6.8 (5.3)	17.3 (2.7)*	26.0 (3.1)*†	5628 (66)*

HS = hypertonic saline.
* Significantly different from the control day.
† Significantly different from 3% HS ($p = 0.002$).

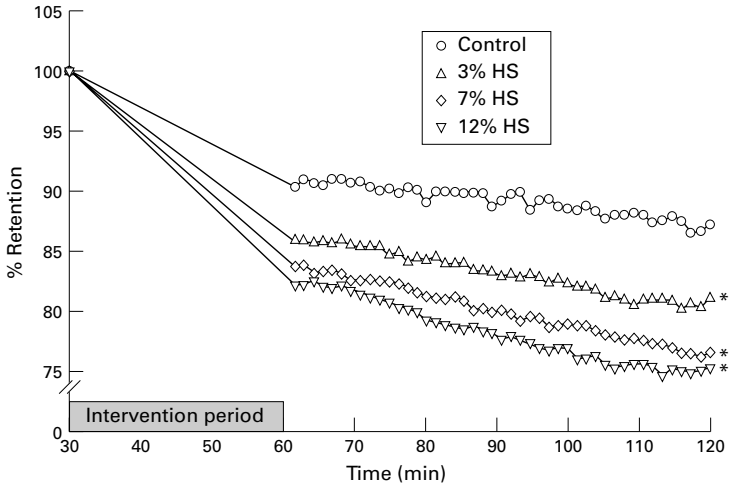

Figure 1 Mean clearance curves for the whole right lung. Curves marked with an asterisk (*) are significantly different from the control day. The amount of activity remaining in the lung at the end of the baseline measurement has been standardised to 100% and the clearance curves refitted to the transformed data set.

the radioaerosol. Baseline clearance was then collected for 30 minutes. The patient then inhaled the intervention solution before being repositioned under the gamma camera for a further 60 minutes for the post-intervention clearance measurement. Spirometric measurements were performed immediately before the intervention, five minutes after the end of the intervention, and at the end of the study (70 minutes after the end of the intervention).

MEASUREMENT OF MUCOCILIARY CLEARANCE

Mucociliary clearance was assessed using the methods previously described by our group.[1–6] The amount of activity cleared from the lung during the baseline measurement, intervention period (%C30) and post-intervention period (%C90) was calculated from the generated activity-time curves. The mucociliary clearance rate was defined in terms of the area under the activity-time curve (AUC). The lower the value of the AUC, the more rapid the mucociliary clearance rate. Comparison of interventions was performed using a one or two factor repeated measures analysis of variance (ANOVA). The Fisher PLSD test was used for the post-hoc analysis.[7]

HYPERTONIC SALINE SOLUTIONS

The study was of a randomised, four way, crossover design. Different doses of hypertonic saline (HS) were achieved by varying the concentration in the nebuliser bowl whilst keeping all other delivery parameters constant. The solutions used were 3%, 7%, and 12% HS. A combination of normal (0.9%) saline and voluntary cough was used as a control. On this day we aimed to have the patients voluntarily cough a number of times that equalled or exceeded the number of coughs on any of the other study days. The coughs were evenly dispersed throughout the inhalation period.

DELIVERY SYSTEM FOR HYPERTONIC SALINE

All intervention solutions were delivered via a modified Omron-NE-U06 ultrasonic nebuliser (Omron NE-U06; Omron Corporation, Tokyo, Japan). This nebuliser has a high volume output (1.5 ml/min) and a small dead volume (0.5 ml). The MMAD of this delivery system was 3.7 μm and 73% of the particles were in the respirable range (Malvern Mastersizer-X, Malvern, UK). The nebuliser was charged with 7.0 ml of the trial solution and inhaled to dryness using a tidal breathing pattern.

Results

BASELINE

There was no significant difference in baseline characteristics (lung function, radioaerosol deposition or baseline mucociliary clearance, $p = 0.4$) on any of the study days. Baseline clearance was reproducible within patients on all study days ($p = 0.1$). Baseline clearance at 30 minutes was significantly impaired in nine of the 10 patients with a mean clearance of 8% (95% CI 4.5 to 12.1) compared with normal subjects in our laboratory (20% (95% CI 12.6 to 26.5), $p<0.01$).

POST-INTERVENTION CLEARANCE

The mean values for percentage clearance of radioisotope at 30 minutes (%C30) and 90 minutes (%C90) after intervention and mucociliary clearance rate (AUC) are shown in table 1. Figure 1 shows the standardised mean activity-time curves after the amount of radioactivity remaining at the end of the baseline acquisition had been standardised to 100%. There was a significant increase in the amount of activity cleared from the right lung at 30 and 90 minutes with all concentrations of hypertonic saline compared with control ($p = 0.002$). In addition there was a significant increase in %C90 for 12% HS solution compared with 3% HS solution ($p = 0.001$). The mucociliary clearance rate (AUC) was significantly increased for the 7% HS and 12% HS compared with control ($p = 0.02$).

The change seen in %C90 for all concentrations of hypertonic saline was not solely due to that at %C30 – that is, there was an ongoing effect beyond the immediate inhalation period for 3% HS (6.2 (0.9)), 7% HS (8.0 (1.7)), and 12% HS (8.7 (1.2)) compared with control (3.9 (3.0); $p = 0.01$). The actual number of patients achieving the highest clearance

on 12% HS (n = 6) was significantly more than expected (n = 2.5) under the null hypothesis of each intervention having an equal probability (one group χ^2 test, p = 0.0001). Three of the remaining four patients did best on 7% HS.

The observed changes in mucociliary clearance were not entirely due to the cough induced by the intervention solution as the number of coughs recorded on the control day (172 (95% CI 135 to 209)) was greater than on any of the other intervention days (3% HS 93 (95% CI 62 to 124), 7% HS 131 (95% CI 95 to 168), 12% HS 167 (95% CI 118 to 216)).

There was a small mean percentage fall in FEV_1 immediately after inhalation of 7% HS (−3.7% (95% CI −9.0 to 1.5)) and 12% HS (−4.9% (95% CI −8.5 to −1.3)) solutions compared with control (1.5 (4.8)%). However, this was not statistically or clinically significant (p = 0.5). This fall in FEV_1 had been reversed by the end of the study – that is, an hour later – resulting in a small (5.4% (95% CI −1.1 to 11.9)) increase in FEV_1 compared with the pre-intervention values. Several patients complained of irritation at the back of their throat with the 12% HS solution.

Discussion

This study shows that mucociliary clearance is enhanced by the inhalation of hypertonic saline in patients with cystic fibrosis. The improvement occurred with all the concentrations of hypertonic saline examined in this study and the effect appears to be dose dependent. As both isotonic saline[6] and cough[8] have previously been shown to improve lung clearance in normal subjects, the enhanced clearance seen in this study with hypertonic saline was in addition to any increase that is likely to have occurred from the inhalation of isotonic saline in combination with voluntary cough. There was no significant airway narrowing when hypertonic saline was inhaled following premedication with a bronchodilator.

Hypertonic saline is known to have a favourable effect on mucus rheology. Dasgupta *et al*[9] have examined the effect of 3% HS compared with normal saline on mucus viscoelasticity in vitro. They reported that hypertonic saline reduced spinnability and sputum rigidity. In addition, they showed that hypertonic saline improved mucus clearability in vitro more than rhDNase (25 μg/ml).

Ziment[10] has postulated that hypertonic saline breaks the ionic bonds within the mucin gel, thereby reducing the effective degree of cross linking and entanglements and lowering the viscosity and elasticity. With chronic infection the mucin macromolecules develop fixed negative charges, resulting in a net repulsion. Hypertonic saline raises the ionic concentration sufficiently to cause a conformational change by increased shielding of the excess negative charges and limiting repulsion. The result is thought to be a more compact structure of the mucus molecule that leads to more effective clearance. This effect is in addition to any effect that may be induced by the osmotic flow of water into the airways, or the stimulating effect of hypertonic saline on cilial beat via the release of prostaglandin E_2.[11]

Wills *et al*[12] have studied the transportability of sputum from patients with cystic fibrosis using a mucus depleted bovine tracheal model. They measured the intrinsic transportability of bovine mucus and compared this with the transportability of expectorated sputum. The relative transportability of cystic fibrosis sputum was reduced at baseline but improved from 33 (3)% to 57 (5)% (p = 0.001) following overnight incubation with solid NaCl (0.5% w/w). In their model, ciliary beat frequency was constant over a wide range of ionic concentrations. They postulated that the observed increase in transportability was due solely to changes in sputum rheology. Thus, there is now good evidence both in vivo and in vitro that mucus rheology is improved with hypertonic saline and this leads to an increase in mucociliary clearance.

Considerable controversy currently exists on the nature of the ionic composition of the airway surface fluid. Several investigators believe the airway surface fluid in patients with cystic fibrosis to be hypotonic compared with normal subjects,[13] whilst others have found it to be hypertonic.[14] If the airway surface fluid in patients with cystic fibrosis is in fact hypotonic, it would seem logical that nebulisation of hypertonic saline may go some way to restoring the ionic imbalance. Due to rapid osmotic regulation[15] this change is likely to be transient and may account for the relatively short effect of hypertonic saline seen in this and other studies.[6 12]

The effect of hypertonic saline on airway defences is also unclear. Mizgerd *et al*[16] have shown that in a low sodium environment (within the range that is typical of cystic fibrosis sputum) the ability of human peripheral neutrophils to phagocytose and kill *Pseudomonas aeruginosa* is significantly reduced. By restoring the sodium concentration to normal, neutrophil phagocytosis and killing was returned to normal. On the other hand, Smith *et al*[17] and Goldman *et al*[18] have recently shown that increased salt concentrations deactivate β-human defensin-1. Defensins are believed to be responsible for the early elimination of bacteria, fungi, and viruses from the airway. Once this primary line of defence has been violated a more vigorous and sustained immune response is evoked, with the migration of large numbers of neutrophils and macrophages into the airway lumen. Any effect of hypertonic saline on defensins is likely to be more important early in the disease process in delaying the onset of colonisation. Once suppurative disease is established, the beneficial effect of hypertonic saline in debulking the airways of copious amounts of viscous secretions, and the pathogens and degradative enzymes contained therein, is likely to be more significant. It is also likely that any effect of hypertonic saline on the ion concentration of the airway surface fluid, and hence on defensin deactivation, would soon be counteracted by the rapid influx of water into the airway lumen along its osmotic gradient.[15]

Hypertonic saline is known to be a bronchoprovocative agent. Rodwell and colleagues[19] have reported the effect of hyperosmolar (10%) saline challenge in 23 patients with cystic fibrosis. All patients were selected because they carried a concurrent clinical diagnosis of asthma. In this study 30% of the patients showed a greater than 15% fall in FEV_1. A second group of patients (40%) showed a transient decrease in FEV_1 with a partial spontaneous recovery before the challenge finished. A third group (30%) showed spontaneous recovery during the challenge with the final FEV_1 recorded at the end of the challenge being significantly higher (4.5%) than immediately prior to the challenge. All groups had a further significant increase in lung function after administration of a bronchodilator at the completion of the challenge. It is expected that the percentages of patients with cystic fibrosis who show a fall in FEV_1 and the magnitude of such a fall would be considerably less in the general cystic fibrosis population.

In the current study all patients were premedicated with a standardised bronchodilator 50 minutes prior to inhalation of the intervention solutions. There was a small (<4.9%) non-significant fall in FEV_1 immediately after inhalation of the 7% and 12% hypertonic saline solutions. This had reversed by the end of the study. In addition, there was no significant change in visual analogue scale (VAS) for patient perception of chest tightness, difficulty in breathing, or wheeze. However, several of the patients complained of significant oropharyngeal irritation with the 12% hypertonic saline solution. The authors believe that 12% hypertonic saline is likely to be the upper limit of tolerability for most cystic fibrosis patients using high volume output nebulisers such as the Omron.

The improved clearance seen with hypertonic saline in this study appears to last for approximately 90 minutes. In a clinical setting it is likely that the nebulisation of hypertonic saline would be limited to twice daily. It is not known what long term impact this more effective, but somewhat transitory, removal of purulent secretions would have on morbidity and mortality. However, Eng and coworkers,[20] in a parallel controlled study, have examined the effect of a two week course of twice daily 10 ml × 6% hypertonic saline on lung function in a group of 52 patients with cystic fibrosis. They found a 15.0 (16.0)% improvement in FEV_1 in the treated group compared with a 2.8 (13.1)% improvement with normal saline ($p = 0.004$). The patients who received hypertonic saline also had a subjective improvement in the effectiveness of chest physiotherapy, quality of sleep, and exercise tolerance assessed on a visual analogue scale. There was no difference in reported adverse events between the two groups and the authors state that treatment with 6% hypertonic saline was well tolerated.

In conclusion, there is now considerable preliminary evidence for a beneficial effect of hypertonic saline in cystic fibrosis. This comes from radioaerosol studies of mucociliary clearance, in vitro studies of mucus rheology and transportability, and a short term clinical trial. Further studies are required to exclude fully any potential undesirable effects on airway inflammation or unfavourable effects on host defence mechanisms. Long term studies of clinical safety and efficacy are also required. The role of hypertonic saline may prove to be short term in enhancing clearance of secretions alone or in combination with physiotherapy. However, the possibility remains that it could be of long term clinical benefit in reducing morbidity and mortality. This needs to be assessed in a long term clinical trial.

This work is supported by the National Health and Medical Research Council of Australia and the Australian Cystic Fibrosis Research Trust.

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