





Acute and durable effect of inhaled hypertonic saline on mucociliary clearance in adult asthma

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ABSTRACT

Background: Impaired mucus clearance and airway mucus plugging have been shown to occur in moderate–severe asthma, especially during acute exacerbations. In cystic fibrosis, where airway mucus is dehydrated, it has been shown that inhaled hypertonic saline (HS) produces both acute and sustained enhancement of mucociliary clearance (MCC). The current study was designed to assess the acute and sustained effect of inhaled 7% HS on MCC in adult asthma.

Methods: Well-controlled, moderate–severe female asthmatic patients (n=8) were screened with a single test dose of albuterol (four puffs by metered-dose inhaler) followed by HS (7% sodium chloride, 4 mL using PARI LC Star nebuliser). Spirometry was measured pre-treatment and 5 and 30 min post-treatment for safety. MCC was measured using γ -scintigraphy on three separate visits: at baseline, during inhalation and 4 h after a single dose of HS.

Results: MCC was acutely enhanced during HS treatment; mean \pm SD clearance over 60 min of dynamic imaging (Ave60Clr) was 8.9 \pm 7.9% (baseline) versus 23.4 \pm 7.6% (acute HS) (p<0.005). However, this enhancement was not maintained over a 4-h period where post-HS treatment Ave60Clr was 9.3 \pm 8.2%. In this small cohort we found no decrements in lung function up to 30 min post-treatment (forced expiratory volume in 1 s 97.4 \pm 10.0% predicted pre-treatment and 98.9 \pm 10.7% predicted 30 min post-treatment).

Conclusion: While MCC was rapidly enhanced during 7% HS treatment there was no effect on MCC at 4 h post-treatment. While these findings may not support aerosolised HS use for maintenance therapy, they do suggest a benefit of treating acute exacerbations in patients with moderate–severe asthma.



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Acute inhalation of 7% saline dramatically enhances mucociliary clearance in adult asthmatics, but, unlike in cystic fibrosis, does not provide sustained enhancement 4 h post-treatment
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Introduction

Mucociliary clearance (MCC) is a critical innate defence to remove mucus and associated toxins, bacteria, viruses and inflammatory cells/cytokines from the lungs. *In vitro* studies have demonstrated that well-differentiated airway epithelial cultures lose their ability to transport mucus as they become progressively dehydrated, thus providing a key mechanistic link between mucus dehydration and defective MCC *in vivo* [1]. Hypertonic saline (HS) has been shown to enhance hydration of mucus and improve mucus rheology *in vitro* [2–4]. *In vivo* inhaled HS acutely enhances MCC in healthy volunteers as well as those with asthma and cystic fibrosis (CF) [3, 5–9]. Moreover, in CF the enhancement is both acute and durable, lasting ≥ 4 h with a single dose and 8 h after repetitive dosing [3, 10] and is associated with improved lung function over as little as a 2-week treatment period [3]. However, in healthy subjects the stimulatory effect is acute and paradoxically slowed after 4 h [7]. Additionally, our recent clinical study in patients with chronic bronchitis showed a general slowing of MCC over a 2-week treatment period [11], and a similar lack of sustained HS effects.

Airway mucus plugging and acute slowing of MCC during exacerbations is a key feature of asthma and contributes to the morbidity and mortality associated with the disease [12–14]. Increased MUC5AC/MUC5B ratios and associated disulfide bonds in airway mucus [15] as well as ciliary dysfunction [16] are features of severe asthma that probably play a role in reduced MCC. However, there is also evidence for increased dehydration of mucus obtained from patients with asthma. LOUGHLIN *et al.* [17] found that induced sputum recovered from asthmatics under baseline (nonexacerbated) conditions had 2.2% solids compared to 1.6% in healthy volunteers, with solids percentage being related to neutrophilic content of sputum. While this level of dehydration in asthmatics is less than that observed in CF [18], they do overlap with solids percentage, as we reported in chronic bronchitis [1]. Furthermore, a single treatment with inhaled dry-powder mannitol, a hyperosmolar therapy, in asthmatics showed significant improvement in sputum rheology [19]. These findings support the rationale and potential for the use of inhaled hypertonic saline to treat reduced MCC in asthma.

Because the clinical benefits from inhaled HS probably depend on the duration of this effect on MCC, assessment of MCC at later time points post-HS inhalation will help determine how this treatment may best be applied. In this current open-label pilot study we assessed both the acute (immediate) and durable (4 h post-treatment) efficacy of HS for improving MCC in patients with well-controlled moderate–severe asthma. Finally, due to concerns of airway hyperresponsiveness in these patients, we pre-treated patients with a bronchodilator (albuterol) and monitored lung function before and after HS treatment.

Study design/method

Study subjects

Adult asthmatics aged 20–53 years with well-controlled moderate–severe asthma at enrolment were studied (defined by step ≥ 3 therapy or by degree of impairment secondary to asthma, per the National Asthma Education and Prevention Program expert panel report [20]). Participants were excluded if forced expiratory volume in 1 s (FEV_1) $< 70\%$ predicted without use of short-acting bronchodilator (albuterol) in the previous 8 h. All participants were on a combination long-acting β -agonist/steroid medication (Advair, Symbicort or Breo) and self-reported adherence to their medication as prescribed during their entire study. All participants were stable, *i.e.* no signs of exacerbation, when studied.

Study design

Participants had a baseline MCC assessment by γ -scintigraphy imaging over a 2-h period following inhalation of radiolabelled particles (technetium-99m sulfur colloid) as described in detail previously [7, 21] (figure 1, supplementary videos). At the conclusion of the 2-h baseline MCC assessment subjects were screened for bronchial hyperreactivity associated with albuterol/HS treatment. 15 min after pre-treatment with four puffs of albuterol (90 μ g each *via* metered-dose inhaler (MDI) with spacer), subjects received a screening dose of HS inhalation by PARI LC Star nebuliser (4 mL 7% sodium chloride inhaled until sputter). Spirometry was measured at 5 and 30 min after completion of HS inhalation. Participants with a 10% fall in FEV_1 that persisted for ≥ 30 min were excluded from further study.

At least 1 week after the screening/baseline MCC visit, we assessed the acute effects of HS on MCC (figure 1). Immediately following the initial 4-min deposition scan of the MCC dynamic imaging, subjects received albuterol and 7% HS inhalation over a 25-min period (as described earlier for screening visit) while being scanned for MCC through the 2-h period (figure 1). Cough frequency was monitored during the measure of MCC. After ≥ 1 week, participants returned to assess the durability of albuterol/HS on MCC. Subjects again received albuterol pre-treatment and HS inhalation as described earlier, but the radioaerosol inhalation and MCC measurement began 4 h later. For each MCC measurement, subjects returned the following day for a 30-min image to assess retention at 24 ± 4 h after initial deposition.

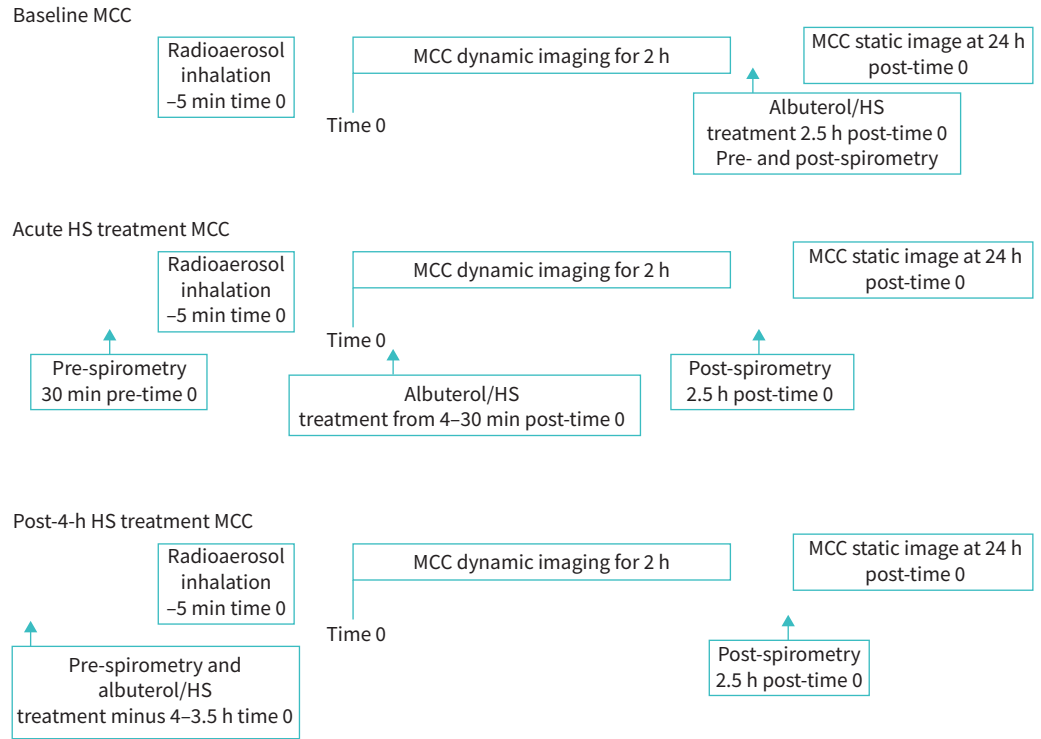


FIGURE 1 Study design. MCC: mucociliary clearance; HS: hypertonic saline.

Baseline MCC measured in a control group of healthy non-asthmatic female adults (n=19) who participated in previous [7, 21] and current studies were included for comparison to baseline MCC in the asthmatic adults.

MCC analysis

By computer analysis, a rectangular whole and central region was circumscribed over the right lung defined by a cobalt-57 transmission scan in each subject [7]. The region was used to determine whole-lung retention (Rt) (decay- and background-corrected) as a fraction of the initial counts over the 2-h imaging period. To describe each retention *versus* time dataset (e.g. mean data shown in figure 2), the

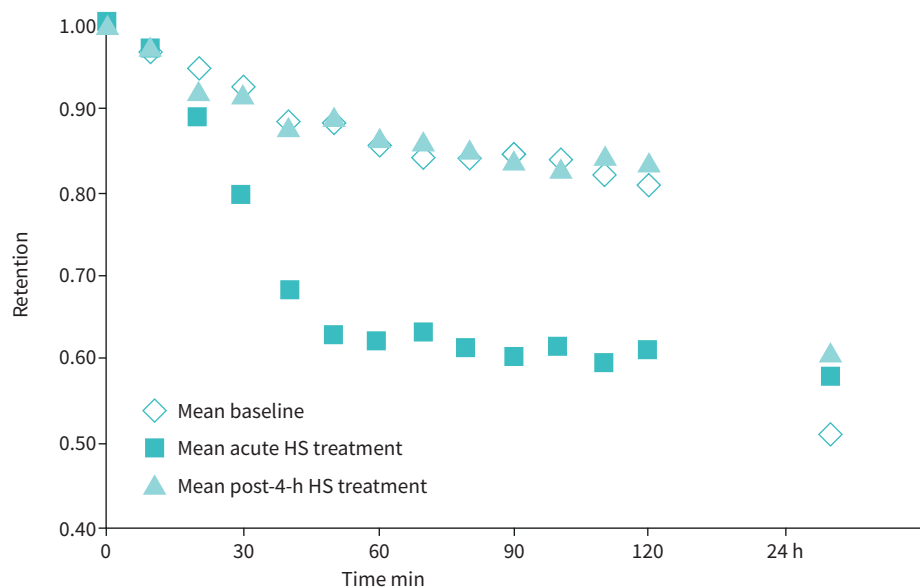


FIGURE 2 Mean retention *versus* time for the baseline, acute hypertonic saline (HS) treatment, and post-4-h HS treatment for eight adult female asthmatics.

average percentage clearance (or $100 \times (1 - Rt)$) over the 60- and 120-min period of observation were computed (*i.e.* average of the 10-min clearance values from 10 to 60 or 120 min). These computed values are signified as Ave60Clr and Ave120Clr, respectively. This method for characterising nonlinear retention *versus* time data over a given period (figure 2) allows for all 10-min datapoints to be considered, in our case area under the curve expressed in terms of average clearance [22, 23] over that period. Regional deposition, a potential confounder of MCC measurements, was also determined by central/peripheral (C/P) ratio for each measure of MCC. Increasing C/P is associated with an increase in average clearance [22, 23]. The retention at 24 h was compared between study visits (figure 2) as an index of initial, regional deposition of radioaerosol between bronchial and alveolated airways [22].

MCC in the healthy control group was only compared through 60-min imaging (*i.e.* Ave60Clr) due to interventions post-60 min in some of these healthy control studies.

Statistics

The pre-specified primary study outcome was whole lung AveClr60 between baseline *versus* acute *versus* 4-h post-challenge visits. Secondary outcomes included changes in spirometry and other MCC outcomes (AveClr120, C/P, 24-h retention). From previous studies we have seen that the distribution of MCC outcomes is normal [3, 7, 11]; we did not expect strong outliers even under the conditions of low numbers.

Data were analysed using a repeated-measure ANOVA using visit as the primary fixed factor (baseline, acute HS, durability HS). Multiple comparisons between visits were then adjusted using Tukey. Effects of C/P and FEV₁ were analysed by adding them as covariates in the model. The relationship between specific outcomes were determined by Pearson's correlations at each visit. Comparisons between asthmatic and healthy subjects were made with independent t-tests. p-values <0.05 were considered significant. All statistical analyses were conducted utilising SAS v9.4 and JMP Pro 14 (SAS, Cary, NC, USA) statistical software.

Study approval

The study was approved by the University of North Carolina institutional review board, and subjects provided written informed consent. The study was registered at Clinical Trials.gov (NCT03556683).

Results

Nine (eight females, one male) asthmatic patients completed the study as designed. Due to the low number of males recruited to the study before interruption by the coronavirus disease 2019 pandemic, we narrowed our findings reported here to female subjects (n=8) including MCC comparison to healthy nonasthmatic females (n=19). No participants were excluded based on the HS screen. Mean±SD age was 29.6±12.1 years and 27.7±6.6 years for asthmatics and nonasthmatics, respectively. On the screening study day, the FEV₁ normalised as a percentage of the predicted FEV₁ value was 97.4±10.0% pre-HS treatment and 97.5±12.2% and 98.9±10.7% at 5 and 30 min post-HS treatment, respectively (nonsignificant).

Figure 2 shows the significantly different mean retention in time data for the three MCC measurements in the female asthmatics (p=0.0017). The acute challenge with HS produced an immediate, rapid clearance of the radiolabelled particles compared to baseline (see supplementary videos), Ave60Clr 8.9±7.9% (baseline) *versus* 23.4±7.6% (acute HS) (p<0.005). In addition, over the entire 120-min period of MCC measurement the average clearance was different between the three MCC measures; greater for acute HS compared to baseline, Ave120Clr 12.8±8.8% (baseline) *versus* 31.1±8.6% (acute HS) (p<0.002). It was evident that only a small fraction of particles were still available for clearance between 60 min and 24 h for the acute HS case. By contrast, there was no effect of HS on MCC measured 4 h post-challenge compared to baseline, Ave60Clr 9.3±8.2% and Ave120Clr 12.4±8.6% for 4 h post-challenge. The 24-h retention was not different between the baseline, acute HS and 4 h post-HS study visits (p=0.14); 0.51±0.06, 0.58±0.09 and 0.61±0.13, respectively.

Regional deposition (C/P) was not different between the three MCC measurements: C/P 2.2±0.5 (baseline), 2.1±0.4 (acute HS) and 2.1±0.4 (4 h post-HS). Both Ave60Clr and Ave120Clr were correlated with C/P for the baseline MCC measurements (R=-0.84, p<0.01 and R=-0.87, p<0.01, respectively). Pre-FEV₁ was not a significant covariate for either Ave60Clr or Ave120Clr. Spontaneous cough only occurred for the acute HS challenge in six of the eight asthmatics during the MCC measurement and was minimal (four or fewer coughs for any subject, mean coughs 2.3 for all).

Baseline MCC trended slower in the small group of female asthmatics compared to the nonasthmatic female cohort, Ave60Clr 8.9±7.9 *versus* 13.9±6.5% (p=0.09), respectively, while neither C/P 2.2±0.2 nor 24-h retention 0.51±0.10 for healthy nonasthmatics was different from the asthmatics.

Discussion

As expected, the acute inhalation of HS in these female asthmatics enhanced MCC compared to baseline. The immediate and very rapid effect was somewhat unexpected, especially without the accompanied coached coughing that is included in HS inhalation/sputum induction protocols [24, 25]. Despite this dramatic acute effect, MCC was unaffected 4 h post-treatment relative to baseline. This is in contrast to findings in CF, where we found enhanced MCC at 4 h post-HS inhalation, similar to that observed acutely relative to baseline [10]. Unlike healthy volunteers, MCC (Ave60Clr and Ave120Clr) was unchanged between baseline and 4 h post-treatment, suggesting that the resident mucus had not been depleted (compromised) by the acute HS treatment 4 h earlier [7], at least in the larger bronchial airways. Our recent study on the effects of HS treatment in chronic bronchitis showed slowing of MCC relative to baseline after multiple treatments over a 2-week period [11].

Whether a longer-term effect may also occur in asthma is uncertain. It may also be that enhanced MCC could have been observed at earlier times post-treatment (*e.g.* 2 h). The dramatic, acute effect may be beneficial where acute mucus clearance is needed, *e.g.* acute exacerbations, where there may be severe slowing of MCC [13, 14] and where in-patient treatment will allow monitoring of any HS-induced hyperreactivity. This is analogous to the indications for use of established, quick-relief medications for asthma (such as albuterol). While the absence of a durable HS effect in these asthmatics may not support its use for maintenance therapy, it may still be useful for asthmatics in whom periodic clearance of mucus may be beneficial. It will be important to consider the safety and duration of effect of HS when exploring potential uses for asthma.

We included MDI albuterol treatment prior to all HS treatment in the current study to mitigate potential airway hyperreactivity in these participants. Therefore, it should be emphasised that any treatment effects we observed on MCC were for the combination of MDI albuterol followed by nebulised HS. β -agonists delivered by MDI have been shown to acutely enhance MCC in asthma [26, 27], but not to the extent observed in the present study. In fact, in one case, no stimulation of MCC was observed despite the fact that delivery of terbutaline by MDI produced significant bronchodilatation [28]. These studies would suggest that a greater dose of β -agonist is required for enhancement of MCC than is needed to induce bronchodilation. Our study design did not allow for distinguishing the relative contribution of HS *versus* albuterol on MCC, but it is clear that any HS treatment will always include pre-treatment with a short-acting bronchodilator to mitigate any HS-induced hyperreactivity.

There was also some very infrequent, spontaneous cough (see supplementary data) associated with the acute treatment; average 2.3 coughs for all. In previous studies in which we incorporated 60 voluntary coughs during the 60–90 min period of MCC measures in healthy adults [23] we found a 70% increase in clearance for MCC *versus* MCC+cough, much less than observed here over the first 60 min (Ave60Clr), a 163% enhancement with only a few coughs. The individual data (supplementary material) showed that even those with no spontaneous coughing had a significant enhancement of MCC with albuterol/HS. While we cannot quantify the contribution of a few spontaneous coughs on clearance in the current study, it nevertheless can be argued that some spontaneous cough associated with the albuterol/HS treatment is advantageous if it aids in the clearance of mucus from the airways.

The single treatment of inhaled 7% HS in our study of adult females with moderate–severe asthma produced no evidence of airway hyperreactivity, *e.g.* coughing or acute reduction in lung function. Retrospective analysis of induced sputum procedures in mild–moderate asthmatics (successive inhalations of 3%, 4% and 5% HS) also showed no adverse effects associated with HS challenge [24]. Nevertheless, the small number of participants in the current study does not allow for adequate power to exclude potential adverse effects from inhaled 7% HS. It may be that a lower tonicity of saline (*e.g.* 3%) would prove to be as effective for acutely enhancing MCC while providing a greater margin of safety compared to 7% HS. Finally, it is not clear whether multiple HS treatments would be well tolerated, but our experience with multiple induced sputum procedures in asthmatics has shown no HS-associated adverse events.

Finally, this pilot study was limited to female adults. While there is some suggestion that healthy males may exhibit slower baseline MCC than females [23], there are no data to suggest gender-based differences for MCC in asthmatics nor differences in HS responsiveness. Therefore, we think it unlikely that further addition of males to the study would change the fundamental findings we observed and report here in females.

Conclusion

MCC was dramatically enhanced during and immediately after acute treatment with inhaled 7% HS delivered by PARI LC Star nebuliser, but had no prolonged effect at 4 h post-treatment. Due to the immediate and short-lived effect of HS on MCC in asthma, we hypothesise that this intervention may be most effective for periodic clearance of mucus or with viral or allergen-induced acute exacerbation where

acute mucus slowing/plugging may be present. Future studies to assess the effect and safety of HS treatment on experimental challenges [14] in these patients may provide support for moving forward with such a treatment regimen.

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