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A Comparison of Sputum Induction Methods: Ultrasonic *vs* Compressed-Air Nebulizer and Hypertonic *vs* Isotonic Saline Inhalation

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SUMMARY **Airway inflammation can be demonstrated by the modern method of sputum induction using ultrasonic nebulizer and hypertonic saline. We studied whether compressed-air nebulizer and isotonic saline which are commonly available and cost less, are as effective in inducing sputum in normal adult subjects as the above mentioned tools. Sixteen subjects underwent weekly sputum induction in the following manner: ultrasonic nebulizer (Medix Sonix 2000, Clement Clarke, UK) using hypertonic saline, ultrasonic nebulizer using isotonic saline, compressed-air nebulizer (BestNeb, Taiwan) using hypertonic saline, and compressed-air nebulizer using isotonic saline. Overall, the use of an ultrasonic nebulizer and hypertonic saline yielded significantly higher total sputum cell counts and a higher percentage of cell viability than compressed-air nebulizers and isotonic saline. With the latter, there was a trend towards squamous cell contaminations. The proportion of various sputum cell types was not significantly different between the groups, and the reproducibility in sputum macrophages and neutrophils was high (Intraclass correlation coefficient, *r* [95%CI]: 0.65 [0.30-0.91] and 0.58 [0.22-0.89], $p < 0.001$). Overall changes in median FEV_1 were small and comparable between all groups. Induction using ultrasonic nebulizers together with hypertonic saline was generally less well tolerated than compressed-air nebulizers and isotonic saline. We conclude that in normal subjects, although both nebulizers and saline types can induce sputum with reproducible cellular profile, ultrasonic nebulizers and hypertonic saline are more effective but less well tolerated.**

Inducing sputum as a means to evaluate airway inflammation is now an established research method in conditions such as asthma[1,2] and chronic obstructive airway disease.[3] It may also have a role in guiding the physicians regarding treatment and management of some airway diseases.[4] The modern method of inducing sputum with hypertonic saline using an ultrasonic nebulizer is shown to be reliable, reproducible and responsive to disease activity and treatment.[2,5,6] Increasingly, emphasis is being placed on making the methodology safer in subjects with unstable airways and also more accessible for wider clinical use.[7,8]

One approach is to employ the use of compressed-air nebulizers and isotonic saline to induce sputum. The common 'compressed-air system' nebulizer is readily available in most hospitals for administering inhalational medicines such as bronchodilator medications to patients with acute exacerbations of airway diseases. Compressed-air nebulizers also cost less than ultrasonic nebulizers. Ultrasonic nebulizers have been shown to be superior to compressed-air nebulizers for particle delivery into the lung in experimen-

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tal settings[9,10-12] and in diseased states.[13-5] However, for the induction of sputum, evidence is lacking that one nebulizer is better than the other.

Although the mechanics are poorly understood, inhaled hypertonic saline may facilitate sputum production by increasing the outward water flux across the airway epithelium, stimulating the cough receptors and, perhaps, the mucociliary escalators.[16] However inhalation of hypertonic saline can cause significant bronchospasms in some asthmatics,[7,17] which has previously been used to indirectly measure bronchial hyperresponsiveness in asthma.[18,19] There is evidence that in asthmatic patients, nebulized isotonic saline is as effective as hypertonic saline in inducing sputum, with a lower tendency to induce bronchospasms.[20,21] Thus, isotonic saline may be used to induce sputum in patients with unstable airways.[22]

In regions with limited healthcare resources such as ours, the possibility of inducing sputum with compressed-air nebulizers using isotonic saline has the potential to encourage a wider use of this method for patients in day-to-day clinical settings. We conducted an open-label study on normal healthy adult subjects to compare a locally available Taiwan-made compressed-air nebulizer, BestNeb, to the low-output ultrasonic nebulizer Medix Sonix 2000 (Clement Clarke, UK), which was previously validated for the purpose of sputum induction.[8] We compared their effectiveness of sputum induction with hypertonic and isotonic saline, and also studied the safety and tolerability of these different approaches.

SUBJECTS AND METHODS

Subjects and study design

Sixteen normal, healthy subjects (mean age ± SD = 27 ± 8 years; 50% male; mean FEV_1 % predicted normal ± SD = 88.0 ± 12.7) were recruited for this study. The subjects were not current cigarette smokers and if they had had smoked previously, had fewer than 5 pack years (one pack year of cigarette smoking is smoking 20 cigarettes [a pack] daily for a year). None had any respiratory symptoms or diseases, nor any other medical illness. Subjects who had respiratory tract infections in the preceding 4 weeks were excluded. After a preliminary screening visit for eligibility, each subject underwent 4 sessions of sputum induction on a weekly basis for 4 weeks, in an open-label fashion. The order in which the sessions were carried out was as follows: ultrasonic nebulizer with hypertonic saline, ultrasonic nebulizer with isotonic saline, compressed-air nebulizer with hypertonic saline, and finally compressed-air nebulizer with isotonic saline. The study was approved by the local university Ethics and Research Committee and informed consent was obtained from all subjects.

Sputum induction

We followed the method originally described by Pin and colleagues.[1] During each session the baseline (pre-induction) FEV_1 was recorded. Subjects then inhaled hypertonic or isotonic saline three times, each time for 7 minutes, using the ultrasonic (Medix Sonix 2000, Clement Clarke, UK) or compressed-air nebulizer (BestNeb, Taiwan). Hypertonic saline was inhaled at an increasing concentration of 3%, 4% and 5%, while isotonic saline (0.9%) was inhaled during all three 7-minute periods. After each 7-minute period of inhalation, the subject would rinse the mouth and blow the nose in order to minimize contamination from saliva or nasal secretions, before expectorating into a sterile container.

Specifications of nebulizers

The compressed-air nebulizer by BestNeb (Taiwan) is a piston-driven compressor, delivering particles with a median size ranging from 0.5 to 5 μm, with an output of 0.2 ml/minute. The ultrasonic nebulizer by Medix Sonix (Clement Clarke, UK) delivers particles of a median size of 5.6 μm, with an output of 0.9 ml/minute. This information was obtained from the product manufacturers. Nebulization in both is administered by inhalation via a mouth piece. For the purpose in this study, both nebulizers were set at their maximum output during the sputum induction.

Sputum processing and examination

We followed the method originally described by Pizzichini and colleagues,[2] where the sputum was selected from saliva and processed within 2 hours. Selected sputum was homogenized by adding four volumes of freshly made 0.1% dithiothreitol (DTT) (Amresco, USA) which was then added to an equal volume of Dulbecco phosphate-buffered saline (D-PBS) (Hyclone, USA). The cell suspension was filtered through a 60 μm nylon gauze (Millipore) and the filtrate centrifuged at 1,185 x *g* for

4 minutes at room temperature. The supernatant was aspirated and stored at -70°C for future use while the cell pellet was resuspended with D-PBS, adjusted to 0.5 x 10^6 cells/ml, placed into cytocentrifuge cups (Hettich, Germany) and centrifuged at 197 x *g* for 10 minutes to make cytoslides. After being air dried, the cytoslides were stained with Leishman's stain (BDH, UK) according to the standard protocol, for a differential cell count on at least 400 non-squamous cells by an independent observer blinded to the subjects' data.

Safety parameters

The FEV_1 was measured prior to each 7-minute session, and the procedure was terminated if the FEV_1 fell below 20%. For sessions using hypertonic saline, if there was fall in the FEV_1 of 10% and 20%, the subsequent induction would be performed with the same concentration of hypertonic saline instead of progressing to a higher concentration as per protocol. The final FEV_1 was then recorded and a rescue β_2-agonist was administered if the FEV_1 fell below 20% or if clinically indicated. The percentage change in FEV_1 was measured in liters with reference to the baseline FEV_1.

Tolerability

After each 7-minute period of inhalation the subjects were asked to score their level of tolerability of the procedure on a numerical scale between 1 (very well tolerated) and 10 (totally unbearable).

Statistical analysis

All data were expressed as median and interquartile range (IQR) and non-parametric tests were used unless otherwise stated. All variables in each subject were first tested for differences using the Kruskal-Wallis test, and if any difference was found, the two paired variables were further analyzed with the Wilcoxon Signed Rank test. The repeatability of the sputum cell types was tested with the intraclass correlation coefficient (ICC) *r*, based on the consistency definition of a one-way random model.[23] A *p* value of ≤ 0.05 was considered significant. All statistical analysis was performed using SPSS™ Version 11.0 for Windows and PrismGraph™ Version 3 for Windows 95 and NT.

RESULTS

All subjects underwent the sputum induction sessions without any serious adverse events. One subject had a 23% fall in the FEV_1 during induction with 4% hypertonic saline using the ultrasonic nebulizer without experiencing any symptoms. This was easily reversed with an inhalatory β_2-agonist.

Measurable sputum cells were obtained in all but one sputum induction session. The unsuccessful induction occurred in one subject while using the compressed-air nebulizer and isotonic saline. Overall, the ultrasonic nebulizer yielded a significantly greater number of sputum cells and a higher percentage of cell viability (Fig. 1). In likely manner, induction with hypertonic saline produced significantly higher total sputum cell counts and a higher percentage of cell viability compared to induction with isotonic saline (Fig. 1). There was a trend towards a lower squamous cell contamination with ultrasonic nebulizer and induction with hypertonic saline compared to compressed-air nebulizer and isotonic saline (Fig. 1).

The proportion of sputum macrophages, neutrophil and lymphocyte cells was not significantly different between the different setups (Table 1). The repeatability for sputum macrophages and neutrophils between the different induction methods was high and statistically significant (*r* [95%CI] for macrophages: 0.65 [0.30-0.91], $p = 0.0001$; for neutrophils: 0.58 [0.22-0.89], $p = 0.0005$). The repeatability for sputum lymphocytes was low and not significant (-0.04 [-0.22-0.44], $p = 0.57$) (Table 1).

Only one subject had an FEV_1 fall of over 20% (i.e. 23%) during inhalation of 4% hypertonic saline via ultrasonic nebulizer and the procedure was halted as per protocol for safety reasons. Another subject had an FEV_1 fall between 10% and 20% (i.e. 11% to 13%) during inhalation of 3% hypertonic saline via ultrasonic nebulizer. He successfully completed the three 7-minute inductions with 3% hypertonic saline as per protocol. Two subjects had FEV_1 falls between 10% and 20% (i.e. 12% and 14%) during inhalation of isotonic saline via ultrasonic nebulizer. Between the different induction methods, the median percentage change in FEV_1 from baseline (pre-induction) was small (less than 3%) and was not statistically significant during each of the three stages. The only exception was between hypertonic and isotonic saline using the compressed-air nebulizer, where the difference in change was significant ($p = 0.008$) (Fig. 2). Induction with isotonic saline using the compressed-

air nebulizer produced a negative fall, whereas hypertonic saline with compressed-air nebulizer produced a positive change.

In general, inductions with hypertonic saline using the ultrasonic nebulizer were less well tolerated compared to isotonic saline and the compressed air nebulizer (Fig. 3). Overall, however, all the induction methods were reasonably well tolerated, with the mean tolerability score in the various stages not exceeding 4 (average 'intolerability' at 5).

DISCUSSION

We have shown that sputum induction with an ultrasonic nebulizer and the use of hypertonic saline yielded a higher total sputum cell count and cell viability than a compressed-air nebulizer and the use of isotonic saline. There was also a trend towards less squamous contaminate with the ultrasonic nebulizer and hypertonic saline. The differential sputum cell counts were comparable irrespective of the nebulizer type or the concentration of saline used. The reproducibility regarding sputum macrophages and neutrophils was high. The different approaches were safe but ultrasonic nebulizer and hypertonic saline were less well tolerated.

We conclude that in normal adult subjects, although both nebulizers and saline types can induce sputum with reproducible cellular profiles, the ultrasonic nebulizer with hypertonic saline are more effective but less well tolerated.

Many studies have compared the effectiveness of ultrasonic versus compressed-air nebulizers in

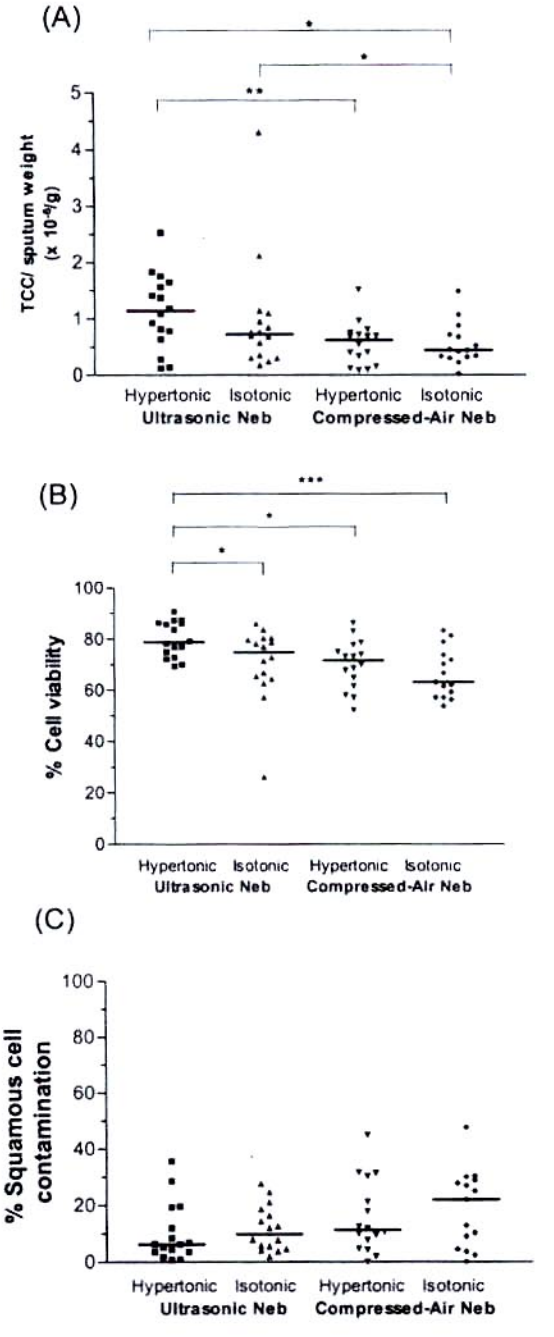

Fig. 1 The effects of induction methods on (A) total sputum cell count (TCC), (B) cell viability and (C) squamous cell contamination. Hypertonic = hypertonic saline; Isotonic = isotonic saline; Neb = nebulizer; * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$ (Wilcoxon Signed Rank test); horizontal bar = median values.

Table 1 The effect of induction methods on the proportion of sputum cell types

	Sputum cell types % (IQR)*		
	Macrophages	**Neutrophils**	**Lymphocytes**
Ultrasonic nebulizer + hypertonic saline	83.2 (69.2 to 93.6)	12.2 (5.6-26.0)	0.5 (0.0-1.2)
Ultrasonic nebulizer + isotonic saline	88.1 (80.7-93.9)	10.2 (5.8-19.2)	0.0 (0.0-1.9)
Compressed-air nebulizer + hypertonic saline	87.0 (75.0-94.6)	12.0 (4.5-24.6)	0.3 (0.0-2.0)
Compressed-air nebulizer + isotonic saline	77.5 (68.2-85.7)	19.0 (12.1-31.2)	1.6 (0.2-2.8)
***p* (Kruskal-Wallis test)**	0.49	0.51	0.34
r* (Intraclass correlation coefficient) (95%CI)*	0.65 (0.30-0.91)	0.58 (0.22-0.89)	-0.04 (-0.22-0.44)
***p* (Intraclass correlation coefficient)**	$p = 0.0001$	$p = 0.00005$	$p = 57$

*Median and interquartile range (IQR) are shown unless otherwise stated
**CI = confidence interval

drug delivery to the lungs in experimental settings[9-12] and in diseased states such as ventilated infants with chronic lung disease,[13] or cystic fibrosis.[14,15] Our study is the first to our knowledge, that compares their effectiveness in inducing sputum for the purpose of evaluating airway inflammation.

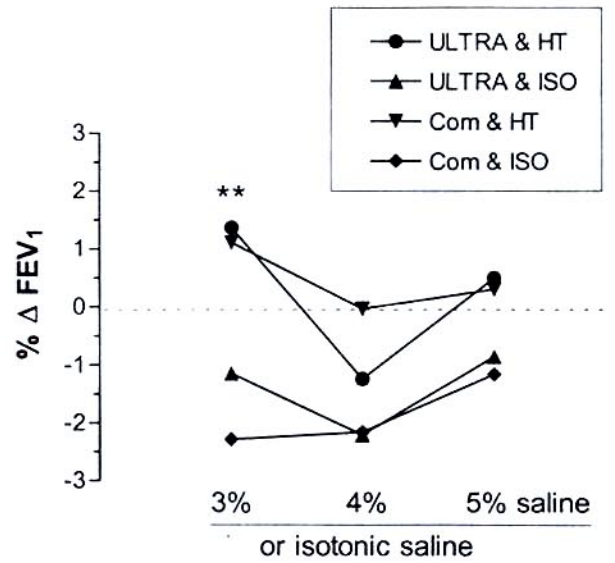

Fig. 2 Percentage change in FEV_1 from pre-induction during the three stages of the different induction methods. Symbols = median values. ULTRA & HT = ultrasonic nebulizer and hypertonic saline, ULTRA & ISO = ultrasonic nebulizer and isotonic saline, Com & HT = compressed-air nebulizer and hypertonic saline, Com & ISO = compressed-air nebulizer and isotonic saline. **$p = 0.008$ Com & HT *vs* Com & ISO (Wilcoxon Signed Rank test).

Compressed-air nebulizers utilize a stream of compressed air or oxygen to draw up liquid through a capillary by the Venturi effect, and to atomize the liquid into respirable particles. Ultrasonic nebulizers focus high-frequency (1-2 MHz) sound waves induced by the vibration of a piezoelectric crystal onto the surface of the liquid in order to create a fountain of droplets. Compressed-air nebulizers have a potential problem in that there is an initial temperature drop that could lead to a reduction in aerosol output as well as solvent evaporation which again could result in an increasingly concentrated drug solution in the reservoir. By contrast, in ultrasonic nebulizers some of the sound wave energy is converted into heat, which warms the aerosol and reduces the concentrating effect. Taken together, ultrasonic nebulizers generally have a higher output than compressed-air nebulizers and mobilize larger particle sizes with diameters typically in the 3.7-10.5 μm

range.[24] The explanation for the better performance of ultrasonic over compressed-air nebulizer in drug delivery is mostly based on this rationale.[9,10,14,25]

Compared to hypertonic saline for sputum induction, inhalation of isotonic saline has shown to produce comparable total and differential sputum cell counts in patients with asthma.[20,21] In addition, the same studies have shown that there is a lesser degree of bronchospasm with isotonic saline. Our study was done on normal, healthy adult subjects, and our findings are similar, although there was a tendency towards higher total sputum cell counts with hypertonic saline (using the same nebulizers), without a significant decrease in lung function. This seems to reiterate the fact that asthmatics are easier irritated by the inhaled hypertonic saline than normal people. As such, isotonic saline inhalation may be safer for sputum induction in asthmatic patients.

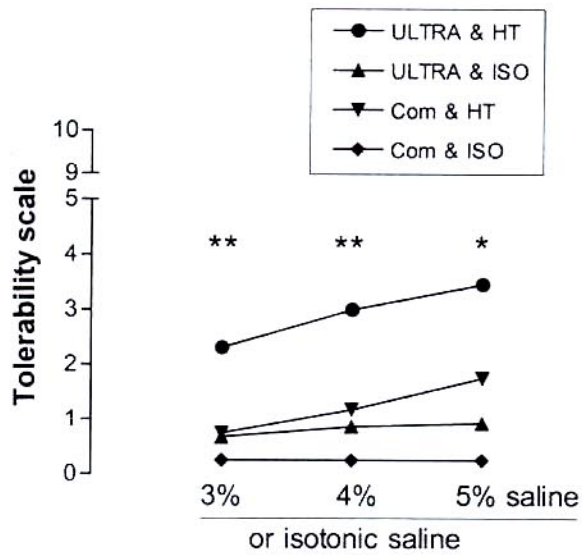

Fig. 3 Subjects' tolerability of the different induction methods. The tolerability scale was measured based on a numerical visual analogue scale between 1 (very well tolerated) and 10 (totally unbearable) during the three stages of induction. Symbols = mean values. ULTRA & HT = ultrasonic nebulizer and hypertonic saline, ULTRA & ISO = ultrasonic nebulizer and isotonic saline, Com & HT = compressed-air nebulizer and hypertonic saline, Com & ISO = compressed-air nebulizer and isotonic saline. $^{**}p = < 0.01$ and $^{*}p < 0.05$. Only p values between ULTRA & HT *vs* Com & HT were shown (Paired t tests).

In this study, the order in which sputum induction was carried out in our subjects was predetermined and not randomly assigned. Furthermore, it was done in an open fashion because of the obvious difficulty in blinding the investigators or the subjects. This has the potential of creating bias in our findings. Although it was not possible to blind the subjects with regards to the type of inhalation saline because of its taste, the investigator might be blinded from this if the saline solution was prepared by a separate investigator, thereby making this a single-blinded study and also allowing the types of inhalation saline be randomly assigned. However, the possibility that the validity of sputum findings might be affected is remote, as each of the induction sessions were separated by one week[2] and the sputum differential counts were conducted by independent blinded observers. The compressed-air nebulizer, BestNeb was chosen based on its availability here, easy portability and low cost. The use of the low-output ultrasonic nebulizer Medix Sonix by Clement Clarke to induce sputum for evaluation of airway inflammation is well-validated,[8] and it was thus chosen to allow us to make a meaningful comparison with the low-output compressed-air nebulizer, BestNeb.

Our findings lend support to the use of compressed-air nebulizers using isotonic saline to induce sputum for the purpose of evaluating airway inflammation in normal adult subjects. Although less effective compared to ultrasonic nebulizers and hypertonic saline, it is well-tolerated, cost effective and convenient to use. However, whether the same can be applied in patients with chronic airway disease or in children should be explored in further clinical studies. The validation of this method may result in its wider use in regions with limited healthcare resources to guide us making appropriate clinical decisions, and in further improving our understanding of airway pathology.

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