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The Tolerability, Safety, and Success of Sputum Induction and Combined Hypertonic Saline Challenge in Children

PETER D. JONES , **ROBYN HANKIN** , **JODIE SIMPSON** , **PETER G. GIBSON** , and **RICHARD L. HENRY**

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Abstract

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Induced sputum using hypertonic saline (HS) is a useful research tool to study airway inflammation (AI). HS provocation testing can also be used to measure airway hyperresponsiveness (AHR). A combined HS challenge and sputum induction procedure has been developed to permit assessment of AI and AHR in a single test. The aim of this study is to report the success and tolerability of sputum induction alone, and in combination with a HS bronchial provocation challenge. Sputum induction alone was performed with β_2-agonist pretreatment. In the combined challenge, no β_2-agonist pretreatment was used. A high-output ultrasonic nebulizer with valve box and tubing were used to deliver 4.5% saline in doubling time periods from 0.5 s to 4 min. Outcomes assessed were completion of the test protocol, adequacy

of sputum samples, decrease in FEV_1, and adverse effects during the procedure. Fifty-three children who underwent a sputum induction alone, and 182 children who underwent a combined sputum induction and bronchial provocation using HS. Sputum induction alone was well tolerated, with 98% of children completing the procedure and only 4% experiencing a significant (> 15%) fall in FEV_1. An adequate sample of sputum was obtained in 92% of children. The combined challenge was completed by 90% of children. A distressing cough occurred in 13% of children and irritation of the mucosa in 1% of children. In the combined challenge an adequate sample of sputum was obtained in significantly fewer children than with sputum induction alone (70% versus 92%, $p < 0.05$). Sputum cellular changes reflected the shorter nebulization time with sputum induction alone. We conclude that induction of sputum using HS after pretreatment with bronchodilator is well tolerated with a high success rate in children. Combining the HS challenge with sputum induction provides additional information and is a useful means of comparing AHR and AI simultaneously, but at the expense of having a reduced success rate in obtaining an adequate sample of sputum, as well as increased side effects.

Asthma is characterized by reversible airways obstruction associated with cough, wheeze, and breathlessness (1). Measuring the degree and severity of airway hyperresponsiveness (AHR) has been an important part of understanding the reversible airways obstruction associated with asthma (2). Measuring AHR has required children to undergo a bronchial provocation involving exercise or inhalation of direct-acting stimuli such as histamine to induce bronchospasm (3, 4).

Over the last decade airway inflammation (AI) has been recognized as a key component of asthma (2). Initial studies characterizing the AI associated with asthma have relied upon the relatively invasive technique of bronchoalveolar lavage (BAL) taken during bronchoscopy (5, 6). Sputum induction using hypertonic saline (HS) has been developed over the last decade, allowing minimally invasive assessment of AI without subjects having to undergo bronchoscopy (7-10). Initially, sputum induction was described using a low-output nebulizer, with children pretreated with β_2-agonist to minimize bronchospasm (7). In many centers a high-output nebulizer is now used to shorten the duration of the procedure (8-10). HS can also be used to measure AHR (11). It is an indirect measure, because HS does not directly cause airway smooth muscle (ASM) contraction. HS causes mediator release from inflammatory cells which then activate ASM. A combined technique of sputum induction with the assessment of AHR using has been described for use in adults (9, 12). The advantage of such an approach is that it permits the simultaneous measurement of AHR and AI with the same noninvasive test. In this approach there is no pretreatment with a β_2-agonist, which means there is a greater potential for bronchoconstriction with a subsequent decline in baseline lung function.

In this study we examine the safety and success of the two techniques for obtaining induced sputum using 4.5% HS in children. We examined the safety and success of the traditional method of induced sputum alone, after pretreatment with β_2-agonist, and compared this with the recently developed technique combining sputum induction with a bronchial challenge procedure.

METHODS

Between 1995 and 1999, 235 children were assessed at the Airways Research Centre (ARC) at John Hunter Hospital in Newcastle, Australia. The children were enrolled in various studies that had approval from the human research ethics committees of Hunter Area Health Service and the University of Newcastle. Informed consent was obtained from the parents or guardian of each child before enrollment.

The children ranged in age from 7 to 16 yr and were required to have a baseline FEV_1 > 70% predicted and be clinically stable. Fifty-three children underwent sputum induction alone. The children who underwent the induced sputum procedure were attending an outpatient asthma clinic at John Hunter Children's Hospital (JHCH) and all had a doctor diagnosis of asthma. One hundred eighty-two children underwent the combined sputum induction and bronchial challenge procedure. These children were recruited from both the outpatient clinics at JHCH and from primary care. This group included 58 healthy children with no history of asthma. All children were studied when they were stable, that is, no reported exacerbation of asthma symptoms or respiratory tract infection in the previous 4 wk. For each patient, a history of recent asthma symptoms was taken and the severity of asthma was classified, based on these symptoms, by two of the authors (P.G. and R.L.H.) as being infrequent episodic, frequent episodic, or persistent asthma using the guidelines from the National Asthma Campaign (1).

Sputum Induction with β_2-agonist Pretreatment

Sputum induction was performed using an inhalation of HS (4.5%) through a mouthpiece and a large two-way valve (Hans Rudolph Inc., Kansas City, KS) connected to a DeVilbiss ultrasonic nebulizer (Somerset) set on the maximum output setting. Spirometry was performed using a computerized spirometry system (Medgraphics, Pulmonary Function System 1070, Series 2; Medical Graphic Communications, Minneapolis, MN). Children performed at least three reproducible expiratory maneuvers. Values were expressed as a percentage of predicted based on normal values (13). The procedure was explained to the child, who would rinse his or her mouth with water to clear debris and squamous epithelial cells. Nose-clips were applied and baseline FEV_1 and vital capacity (VC) were measured. The child then received 200 μg of al through a pressurized metered-dose inhaler with a valved holding chamber (Volumatic; A and Hanbury's, Melbourne, Australia). Spirometry was repeated 10 min later and postbronchodilator value recorded. The child then inhaled 4.5% saline for a period of 30 s. Lung function was repeated 1 min later. If no sputum was obtained and lung function was greater than 80% of the baseline value, the test would continue. The child would then continue inhalation of HS for periods of 1 min, 2 min, and then two periods of 4 min. The child was encouraged to cough up any sputum after each dose of HS. A record of any side effects experienced by the child undertaking the test was made at the end of each elapsed time period. The study would conclude either when the child produced a macroscopically adequate sample of sputum (7) or when the child had completed all five periods of breathing the 4.5% saline or when lung function had dropped below 80% of the baseline value. If the child had a greater than 20% fall in FEV_1, a further dose of albuterol (2 × 100 μg puffs) was administered using a metered-dose inhaler and Volumatic, and recovery monitored.

Combined Hypertonic Saline Challenge and Sputum Induction

In the combined challenge procedure, the major differences in the protocol were that the dose of HS delivered to the patient was recorded (to enable the calculation of the provocation dose) and bronchodilator pretreatment was not used. HS bronchial provocation challenge was performed as described (11, 14) with the addition that sputum was induced by assisted expectoration. Children withheld bronchodilators, antihistamines, and cromoglycate for their duration of action before testing. A record of any side effects experienced by the child undertaking the test was made at the end of each elapsed time period. If the child developed a cough and the challenge test was completed, they would receive treatment with 2 × 100 μg puffs of albuterol delivered by a metered-dose inhaler and Volumatic. If a child had a 20% reduction in FEV_1 recorded, they were treated with 2 × 100 μg puffs of albuterol delivered by a metered-dose inhaler and Volumatic. After 10 min lung function was measured, and the challenge continued. If the lung function remained less than 80% of the baseline value, a second treatment with albuterol was administered. Such children were observed in the pulmonary function laboratory for a further 1 h before being discharged home.

Sputum Processing

We used the methods described by Cai and coworkers (15) to process the sputum. The sputum was initially assessed macroscopically for plugs at the time of collection. An adequate specimen was defined as one producing countable cytospin slides for estimation for differential cell count, with minimal squamous contamination (< 50%) and pulmonary macrophages present (14). The presence of pulmonary alveolar macrophages and a lack of squamous epithelial cells confirmed the lower respiratory tract origin of the sample.

Outcomes

The outcomes assessed were the proportion of children completing each protocol, the properties of subjects producing an adequate sputum sample, the decrease in lung function, adverse effects reported by subjects, and those observed by the technician during the test. Proportions were compared using a two-sided Fisher exact test. Continuous data were a[illegible] using the Kruskal-Wallis test for nonparametric data.

RESULTS

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There were 53 children with asthma, 54% male, who underwent an induced sputum procedure after pretreatment with a bronchodilator. The average age of the children was 11 yr (SD 2.0). Table 1 shows how 52 of the 53 (98%) children completed the sputum induction protocol and 92% (49 of 53) were able to produce an adequate sample of sputum. Four children were unable to produce an adequate sample of sputum. On three occasions there was excessive squamous cell contamination and on one occasion a child was unable to expectorate any sputum. This child found the salty taste of the HS unpleasant and discontinued the test. On two occasions a significant cough was noted by the investigator during the induced sputum procedure. The

cough was not severe enough to cause the test to be stopped, and on both occasions the children were able to produce adequate samples of sputum. The median decrease in FEV_1 was 0.0% (interquartile range [IQR]: 3.5% fall to 6.0% increase). Two children who had persistent asthma experienced a reduction in baseline FEV_1 of greater than 20%. Both responded well to an additional dose of albuterol.

There were 182 children, 58% male, who presented for a combined HS challenge (Table 1). The average age of the children was 10.8 yr (SD 2.0). The combined challenge was completed by 90% (164 of 182) of the children. Eighteen children were unable to complete the HS challenge because of severe cough on 16 occasions, and the child becoming frightened and distressed on two occasions. An adequate sample of sputum was obtained from 70% (127 of 182) of the children who presented for a combined challenge. This group included seven children who produced an adequate sputum sample but who were unable to complete the challenge part of the procedure because of severe coughing. Both an adequate sample of sputum and a completed HS challenge were obtained from 66% (120 of 182) of the children studied.

Of the 164 children who completed the challenge, 13% (21 of 164) did not produce any sample, and 14% (23 of 164) completed the challenge but produced a sample of sputum that was inadequate because of squamous cell contamination. During the challenge a distressing cough was noted in 13% (23 of 182) of children. Treatment with inhaled albuterol was effective in treating the cough in nine of the children who had to stop the challenge because of distressing cough. The median fall in FEV_1 during the combined challenge was 8.0%. The maximal recorded decrease in FEV_1 was 25.0%. All of the 70 children who had a greater than 15% fall from baseline FEV_1 responded to treatment with a bronchodilator and the lung function improved to 93 to 100% of baseline.

Comparison of Techniques

Table 1 outlines a comparison between the outcomes for the induced sputum and combined challenge procedures. Bronchodilator pretreatment resulted in a higher test completion rate (98% versus 90%, p = 0.0002), and a higher success rate in obtaining an adequate sample of sputum (92% versus 70%, p = 0.03). There was no difference between the two protocols i success rate for obtaining adequate sputum in children with persistent asthma, where fo the induced sputum and combined challenge procedure the success rate was the same (versus 94%, p > 0.05). In each of the other categories of asthma, obtaining sputum was more difficult in the combined challenge. A contaminated sample of sputum was more common in the combined challenge group (14% versus 6%, p = 0.02). It was also more common for the children to be unable to produce any sputum in the combined challenge (12% versus 0%, p = 0.004).

Pretreatment with bronchodilator resulted in fewer children having a significant decline in FEV_1 (4% versus 38%, p = 0.001). There were no children who developed symptoms of asthma that required medical attention over the 24 h after completing the test. In both groups there was a small number of children who rejected the nebulizer and mouthpiece and did not want to proceed with the test (2% versus 1%, p = 0.4). Overall, sputum induction was better tolerated in

the group with pretreatment with a bronchodilator. The main side effect experienced was cough. In the induced sputum procedure cough was quite uncommon with only 4% (2 of 53) reporting cough. Both of these children were able to continue with sputum induction until they had produced sputum. This was significantly less than the challenge procedure where 13% ($p = 0.0005$) of the children complained of cough. The cough appeared to be more severe during the combined challenge because only 30% (7 of 23) were able to fully complete the challenge.

Sputum cell counts for total cell count, neutrophil %, lymphocyte %, eosinophil cationic protein (ECP), and interleukin-8 (IL-8) were similar with the two induction methods (Table 2). The sputum quality tended to be less in the sputum induction method but this was not significant. Significant differences were observed in cellular viability, macrophage %, eosinophil %, epithelial %, and HS nebulization time.

DISCUSSION

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This study demonstrates that sputum induction can be performed in children both with and without β_2-agonist pretreatment. Sputum induction with pretreatment with β_2-agonist was significantly more likely to produce an adequate sample of sputum than the combined challenge. The safety of the two procedures was documented through repeated measurements of lung function and careful recording of adverse side effects during sputum induction. Both procedures were well tolerated, however again the sputum induction with β_2-agonist pretreatment was better tolerated than the combined challenge procedure where a severe cough was a significant problem for some children.

Varying the nebulization time during sputum induction can alter the sputum differential cell counts. β-agonists do not alter sputum cell markers (17). Shorter nebulization times are associated with increases in neutrophils and eosinophils with fewer macrophages (18). Longer nebulization times sample cells from progressively lower in the respiratory tract, resulting in fewer neutrophils and eosinophils and increased macrophages. The differences in cell counts seen in this study reflect the shorter nebulization times with the induction-only techniqu
future studies such variations in cellular differentials might be best controlled for by usir
constant nebulizations.

When sputum induction was initially described for use in children with asthma, the technique used β_2-agonist pretreatment and a low-output nebulizer (7). The reported success rate was 76%. A change to a high-output nebulizer meant that a greater dose of saline could be delivered to the airway and resulted in an increase in the success rate to 84 to 92% (9, 14, 15, 19). Because AHR is a key part of asthma, and because HS can also be used to assess AHR, the technique was modified to allow assessment of AHR and sputum induction with the one test (9, 12). This provided the potential to measure simultaneously the two variables of history of AI and AHR.

The combined challenge procedure was well tolerated, and in 90% of the children the test was completed with an adequate sample obtained in 70%. This is consistent with the results of

Wilson and coworkers (10), who reported that 92% of children completed the challenge protocol with an adequate sample obtained in 56%. However, it was more difficult to obtain an adequate sample of sputum using the combined challenge protocol. Twelve percent of children were unable to produce any sputum despite completing the challenge procedure compared with the 2% of children who produced no sputum after sputum induction with preadministration of salbutamol. In a further 14% of children there was excessive squamous cell contamination of the sputum samples, leading to the collected samples being assessed as inadequate after the combined challenge.

One interpretation of these findings is that preadministration of β-agonist makes it easier to obtain secretions from the lower respiratory tract. It may be that the bronchodilation allows a greater dose of HS to reach the lower respiratory tract and hence induce sputum by causing an osmotic gradient for the movement of water into the airway lumen. Albuterol also increases mucociliary clearance, which would permit greater sputum clearance (15). Another interpretation is that there was a selection bias because children who underwent the sputum induction had more severe asthma than those who had the combined procedure. The success rate of obtaining sputum in those children who underwent the combined challenge with persistent asthma was 93%, which is the same as those children who underwent the sputum induction alone. However, the combined challenge was significantly less successful in milder forms of asthma. This research suggests that in children with either mild or no symptoms of asthma, to obtain information about AHR and AI one should consider performing a two-step procedure. Initially one should perform a sputum induction with pretreatment with a bronchodilator followed by a combined challenge to document AHR. In children with more severe symptoms of asthma, it might be possible to perform the combined challenge only.

Large-scale epidemiologic studies seeking to characterize the asthma phenotype in terms of both AHR and AI are more likely to find the combined challenge of benefit. In such circumstances, the resource savings of using a single test would outweigh the missing data that can occur in the combined challenge. However, in clinical trials where AI is the primary outcome, the induction-only method would be preferred.

The main side effect experienced by the children undergoing these procedures was coug
Cough occurred more frequently with the combined procedure and led to the premature
cessation of the test. The mechanism of cough most likely relates to the effect of HS causing release of mediators from mast cells and sensory nerves in the respiratory mucosa, leading to bronchoconstriction and activation of cough receptors. The main safety issue to arise during sputum induction with HS was the development of significant airflow obstruction. Our study shows that both bronchoconstriction and cough are inhibited by the administration of albuterol before breathing HS in the majority of the children. This is most likely by its action as a bronchodilator and its effect on mediator release. However, the protective action of β_2-agonist can be overcome by HS, because there were two children who despite pretreatment with salbutamol experienced a significant reduction in FEV_1 and a further child who developed a significant cough. This highlights the importance of repeated measurements of lung function and delivering the dose of HS in a graded fashion, as outlined in METHODS, in order to ensure the safety of children during sputum induction.

CONCLUSIONS

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In conclusion, both the induced sputum procedure and combined HS challenge and sputum induction procedure were well tolerated by children with asthma. The induced sputum procedure had a higher success rate in obtaining sputum, with that increase being possibly attributed to the preadministration of β-agonist. The combined challenge, with its ability to document AHR, is safe and appears to be a useful test for assessing lung function and obtaining sputum. The results related to the presence and severity of asthma symptoms. A distressing cough is the main side effect, and children and their parents should be warned of this before undertaking the study.

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Correspondence and requests for reprints should be addressed to Dr. Peter D. Jones, Senior Lecturer in Paediatrics and Child Health, University of Newcastle, John Hunter Children's Hospital, New Lambton NSW, 2305, Australia. E-mail: pjones@mail.newcastle.edu.au

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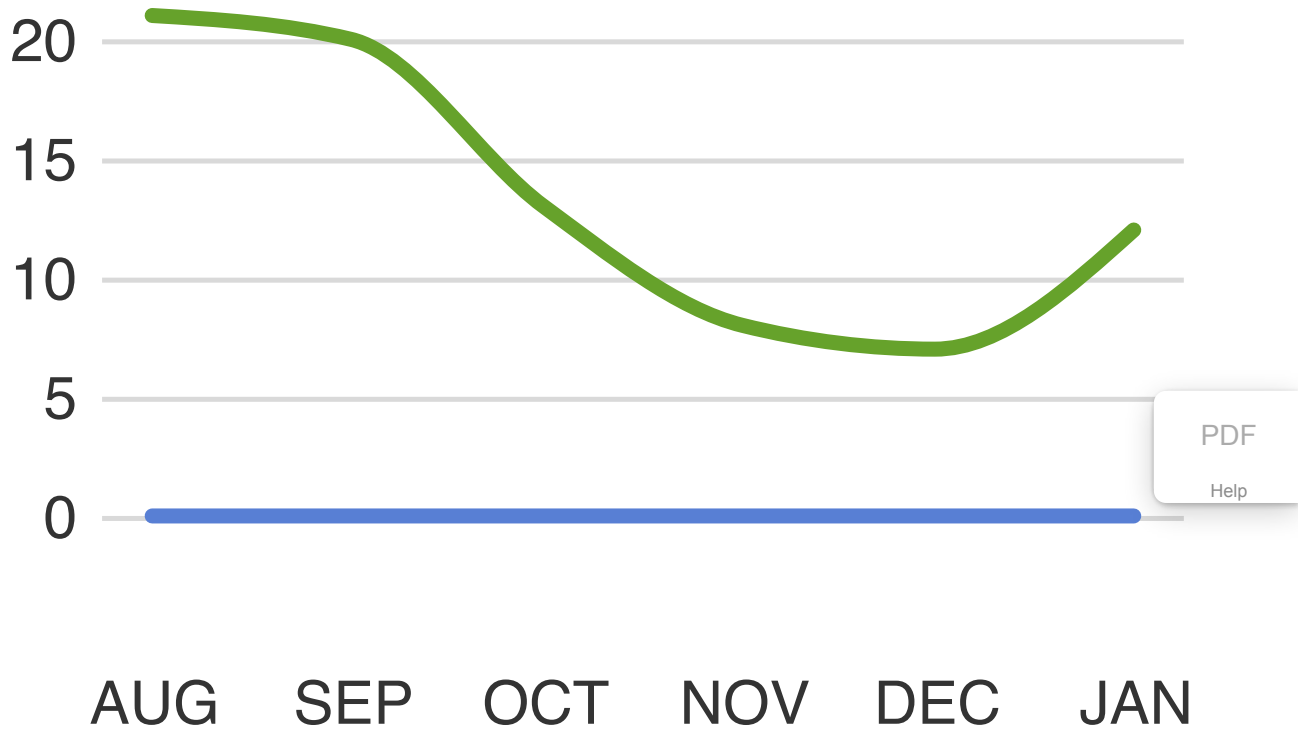

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