Effect of Sputum Induction on Spirometric Measurements and Arterial Oxygen Saturation in Asthmatic patients, Smokers, and Healthy Subjects*

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Background: Sputum production induced by inhalation of hypertonic saline solution has been proposed as a technique to collect secretions and inflammatory cells from the airways of subjects with bronchial asthma or with a history of smoking. The aim of this study was to determine the effect of a sputum induction procedure on spirometric results and arterial oxygen saturation (SaO₂) in asthmatic patients, smokers, and healthy subjects.

Methods: We recruited 14 subjects suffering from asthma (11 men and 3 women; age range, 18 to 49 years), 14 subjects with a history of smoking (5 men and 9 women; age range, 23 to 64 years), and 9 healthy volunteers (7 men and 2 women; age range, 28 to 54 years). To obtain a sample of induced sputum, all subjects inhaled a mist of 3% hypertonic saline solution nebulized for 5 min and repeated the cycle no more than four times. Asthmatic patients were pretreated with 200 µg salbutamol (inhaled). During sputum induction, the transcutaneous SaO₂ was continuously measured and baseline, fall, and the differences between baseline and fall SaO₂ were recorded. Additionally, we measured the duration of mild desaturation (change in SaO₂, < 4%) and of marked desaturation (change in SaO₂, > 5%) in each subject. Finally, baseline FEV₁ and changes in FEV₁ as a percentage of baseline values were recorded in all subjects.

Results: We found that baseline and fall SaO₂ values for the three groups were similar. However, in each group a significant mean change in SaO₂ was evident during sputum production (asthmatic patients, 6.0%; smokers, 5.3%; healthy subjects, 6.0%). Moreover, the mean durations of mild desaturation were 7 min, 21 s in asthma patients; 8 min, 24 s in smokers; and 7 min, 16 s in healthy subjects. Similarly, the durations of marked desaturation were 1 min, 25 s in asthmatic patients, 1 min, 19 s in smokers, and 1 min, 21 s in healthy subjects. The mean (± SD) fall in FEV₁ was not statistically different among the three groups (asthmatic patients, 1.36 ± 5.6%; smokers, 7.56 ± 11.76%; and healthy subjects, 0.05 ± 9.6%). However, one smoker did experience excessive bronchoconstriction (fall in FEV₁, > 20%).

Conclusions: This study demonstrated a significant and comparable fall in SaO₂ during sputum induction by inhalation of hypertonic saline solution in asthmatic patients, smokers, and healthy subjects. The results suggest that subjects who are hypoxemic before sputum induction require SaO₂ monitoring during the procedure.

Key words: asthma; induced sputum; oxygen saturation; spirometric measurements

Abbreviations: PC₂₀ = provocative concentration of substance causing 20% fall in FEV₁; SaO₂ = arterial oxygen saturation

During the past few years, sputum production induced by inhalation of hypertonic saline solution has been increasingly proposed as a noninvasive alternative to bronchoscopy for collecting secretions and inflammatory cells from the airways of subjects with bronchial asthma or a history of smoking.

Although sputum production induced in this way is considered to be a safe investigative procedure, the inhalation of hypertonic saline solution can induce bronchoconstriction in subjects with bronchial hyperreactivity. Therefore, for the safety of asthmatic patients who undergo sputum induction, a protocol for monitoring pulmonary function and administration of a bronchodilator has been proposed. This method of sputum induction (performed according to this protocol) has been shown to be safe even in patients with severe asthma. Also,
the procedure has been well tolerated by smokers with chronic airway obstruction. To date, no studies have evaluated the safety of the sputum induction procedure in smokers.

Sputum production induced by inhaling hypertonic saline solution is known to cause marked arterial oxygen desaturation in HIV-positive patients. However, no studies of the effects of hypertonic saline solution-induced sputum production on arterial oxygen saturation \( (\text{SaO}_2) \) in asthmatic patients or subjects with a history of smoking have been conducted.

The aim of this study was to assess the effects of inhaling hypertonic saline solution to induce sputum production on lung function parameters and \( \text{SaO}_2 \) in a group of subjects with bronchial asthma, a group of smokers, and, for comparison, a group of healthy subjects.

**Materials and Methods**

**Subjects**

We selected a group of 14 patients (age range, 18 to 49 years) with bronchial asthma as defined by the American Thoracic Society. We included lifetime-nonsmoking patients with no respiratory infections or spontaneous asthma relapses in the 4 weeks before the study. Respiratory symptoms were controlled with inhaled \( \beta_2 \)-agonists on a daily basis or as required. Patients requiring steroids or sodium cromoglycate were excluded. Baseline \( \text{FEV}_1 \) had to be > 60% of the predicted value. The presence of atopy was assessed by skin prick tests with a standard battery of eight common inhalant allergen extracts.

We recruited a second group of 14 subjects (aged 23 to 64 years) with a history of smoking of 5 to 80 pack-years. Subjects did not complain of respiratory infections within 4 weeks before the study and were asked not to smoke before sputum induction.

As a control group, we included nine healthy lifetime-nonsmoking volunteers recruited from the hospital staff (age range, 28 to 54 years) who had experienced no acute respiratory illness within 4 weeks before the study. All subjects denied personal or family histories of allergy or respiratory disease.

Each subject gave informed, signed consent, and the study protocol was approved by the Parma Hospital and University of Parma Ethical Committee.

**Sputum Induction Procedure**

Sputum induction was performed between 8:00 AM and 10:00 AM according to a slightly modified version of the Fahy method. All subjects were asked to inhale sterile 3% saline solution that was nebulized from an ultrasonic nebulizer (Heyer Orion 1; Carl Heyer GMBH; Bad Ems, Germany) with a 2 to 2.8 mL/min output range. The reservoir of the device was filled with 100 mL of solution. Inhalation lasted until a reliable sample (at least 2 mL) of sputum was obtained, or was prolonged for a maximum of 30 min. If side effects became evident, the procedure was discontinued. The aerosol was inhaled through a tube that was 85 cm long with a mouthpiece, and the nostrils of subjects were closed with clips to prevent nasal inhalation. Intake of hypertonic saline solution was interrupted every 5 min so that subjects could expectorate sputum into a clean plastic container. Subjects first discarded excess saliva into a separate bowl and thoroughly rinsed their mouths before each expectoration. Subjects were encouraged to cough up secretions at any time during the procedure. Simultaneously, spirometric measurements were taken and expectorated secretions were saved for analysis. The duration of the overall sputum induction procedure was also recorded.

**Lung Function Study**

Spirometric measurements were made on all patients before sputum induction. Then asthmatic patients inhaled two puffs (200 \( \mu \text{g} \)) of salbutamol and spirometric measurements were repeated on these patients 15 min later. Next, all subjects began inhalation of saline solution and, after each 5-min inhalation, spirometric measurements were repeated.

Spirometric measurements were made with a flow-sensing spirometer connected to a computer for data analysis (Vmax 22; SensorMedics; Yorba Linda, CA). Each subject was given careful instruction on how to use the spirometer, and the best \( \text{FEV}_1 \) value of at least three maximally forced expiratory maneuvers was recorded. Falls in \( \text{FEV}_1 \) were measured as a percentage of the baseline \( \text{FEV}_1 \), which was expressed as a percentage of the predicted \( \text{FEV}_1 \). If the \( \text{FEV}_1 \) dropped \( \geq 20\% \) from baseline values after inhalation of hypertonic saline solution, nebulization was discontinued and 200 \( \mu \text{g} \) salbutamol (inhaled) was promptly administered.

On a different day, we measured bronchial responsiveness of all asthmatic subjects with a methacholine challenge test according to a standardized procedure. Each subject inhaled increasing concentrations of methacholine (0.03 to 64 mg/mL) nebulized by a dosimeter (model MB3; MEFAR; Brescia, Italy) with an output of 9 \( \pm 0.3 \) \( \mu \text{L/puff} \), doubling the concentration of methacholine until \( \text{FEV}_1 \) was reduced by 20% from its value after inhaling saline solution. Bronchial response to methacholine was expressed as the provocative concentration causing a 20% fall in \( \text{FEV}_1 \) (\( \text{PC}_{20} \)) and was calculated by linear interpolation between the two final points of the log dose-response curve.

**Oximetry Studies**

In all subjects, \( \text{SaO}_2 \) was continuously monitored from 2 min before the start of the sputum induction until the procedure was completed, and for 5 min after completion, or until recovery of the baseline value. In addition, for the asthmatic group, \( \text{SaO}_2 \) was measured before and 15 min after salbutamol inhalation. \( \text{SaO}_2 \) was monitored with a pulse oximeter (Healthdyne; Marietta, GA). A finger probe was applied to the nondominant hand, and saturation readings were stored in the oximeter memory every 10 s.

For each subject, we recorded the baseline values and the fall in \( \text{SaO}_2 \) percentages. The baseline \( \text{SaO}_2 \) value was the average of the \( \text{SaO}_2 \) readings taken before the start of the sputum induction. The fall in \( \text{SaO}_2 \) was defined as the lowest \( \text{SaO}_2 \) measurement sustained for > 10 s during the procedure. In each subject, the difference between the baseline and maximal fall \( \text{SaO}_2 \) was calculated. To measure the duration of desaturation, in each subject we counted the number of saturation readings showing an \( \text{SaO}_2 < 4\% \) when compared to the baseline value (mild to moderate desaturation), and the number of saturation readings showing an \( \text{SaO}_2 > 5\% \) when compared to the baseline value (severe desaturation).

**Statistical Analysis**

\( \text{FEV}_1 \) values were expressed as a percentage of the predicted value. For methacholine, the \( \text{PC}_{20} \) values were log-transformed.
before analysis. FEV\textsubscript{1} values were presented as mean ± SD, and log-transformed values were presented as geometric mean ± geometric SEM. Differences in numerical data among groups were determined by one-way analysis of variance and the Student-Newman-Keuls test. Differences in qualitative data were analyzed by the Fisher Exact Test. Relationships were estimated by the Pearson correlation test, and \( p < 0.05 \) was considered significant.

**Results**

Subject characteristics are shown in Table 1. Asthmatic subjects showed significantly lower baseline FEV\textsubscript{1} values than subjects with a history of smoking or healthy subjects \( (p < 0.05) \), and no differences were found among the three groups with respect to gender and age. During the sputum induction procedure, the most commonly reported side effects included slight nausea with retching and an unpleasant salty taste. No subject developed side effects sufficiently severe to require premature termination of the procedure.

In asthmatic subjects, baseline FEV\textsubscript{1} values ranged from 70 to 136\% (mean ± SD, 103.6 ± 14.9\%), and their bronchial responsiveness to methacholine ranged from mild to moderate; PC\textsubscript{20} values ranged from 0.41 to 12.24 mg/mL (geometric mean ± geometric SEM, 2.28 ± 1.29 mg/mL). After salbutamol inhalation, the mean FEV\textsubscript{1} value was 114.3 ± 13.6\% \( (p < 0.0001 \), when compared to baseline). After saline solution inhalation, the mean maximal fall in FEV\textsubscript{1} from the post-salbutamol administration value was 1.36 ± 5.6\%.

No subject experienced excessive bronchoconstriction \( (> 20\% \) drop in FEV\textsubscript{1} from postbronchodilator value). Baseline Sa\textsubscript{O\textsubscript{2}} values ranged from 97 to 99\% (mean, 97.8 ± 0.7\%). After saline solution inhalation, the mean values of the fall of Sa\textsubscript{O\textsubscript{2}} and the change in Sa\textsubscript{O\textsubscript{2}} were 91.8 ± 1.7 \( (p < 0.0001 \) when compared to baseline Sa\textsubscript{O\textsubscript{2}}) and 6.0 ± 2.0, respectively (Fig 1). The duration of the overall sputum induction procedure was 24 ± 5 min. Moreover, the duration of the mild-to-moderate desaturation was 7 ± 5 min, 21 ± 14 s (range, 1 min, 20 s to 14 min, 10 s), and the duration of the severe desaturation was 1 ± 1 min, 25 ± 20 s (range, 20 s to 4 min, 10 s).

In subjects with a history of smoking, FEV\textsubscript{1} baseline values ranged from 91 to 154\% of predicted values (mean, 125.1 ± 18.9\%). After saline solution inhalation, the mean maximal fall in FEV\textsubscript{1} from the baseline value was 7.58 ± 11.76\%. One of 14 subjects experienced excessive bronchoconstriction (his FEV\textsubscript{1} fall from baseline value was 20.1\%). Baseline Sa\textsubscript{O\textsubscript{2}} values ranged from 96 to 99\% (mean, 97.85 ± 0.77\%). After saline solution inhalation, the mean percentages of the fall in Sa\textsubscript{O\textsubscript{2}} and the change in Sa\textsubscript{O\textsubscript{2}} were 92.9 ± 1.7 \( (p < 0.0001 \) when compared to baseline Sa\textsubscript{O\textsubscript{2}}) and 5.3 ± 1.5\%, respectively (Fig 1). The duration (mean ± SD) of the overall sputum induction procedure was 23 ± 5 min. Moreover, the duration of the mild-to-moderate desaturation was 8 ± 6 min, 24 ± 23 s (range, 10 s to 20 min, 40 s), whereas the severe desaturation duration was 1 ± 2 min, 19 ± 21 s (range, 10 s to 5 min, 30 s).

In healthy subjects, FEV\textsubscript{1} baseline values ranged from 112 to 168\% of predicted values (mean, 133.2 ± 18.9\%). After saline solution inhalation, the mean maximal fall in FEV\textsubscript{1} from baseline value was 0.05 ± 9.6\%. No subject experienced excessive bronchoconstriction. Baseline Sa\textsubscript{O\textsubscript{2}} values ranged from 97 to 99\% (mean, 97.8 ± 0.5\%). After saline

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*Gmean = geometric mean.
†p < 0.05 vs smoking and healthy subjects.

**Figure 1.** Individual and mean values of baseline Sa\textsubscript{O\textsubscript{2}} (○) and fall in Sa\textsubscript{O\textsubscript{2}} (●) in 14 patients with asthma, 14 subjects who smoked, and 9 healthy subjects during sputum induction procedure.
solution inhalation, mean values for the fall in \( \text{Sa}_2 \) and the change in \( \text{Sa}_2 \) were 92.1 \( \pm \) 2.7 (\( p < 0.0001 \) when compared to baseline \( \text{Sa}_2 \) values) and 6.0 \( \pm \) 3.0%, respectively (Fig 1). The mean duration (\( \pm \) SD) of the overall sputum induction procedure was 24 \( \pm \) 5 min. Moreover, the duration of the mild-to-moderate desaturation was 7 \( \pm \) 6 min, 16 \( \pm \) 18 s (range, 1 to 19 min) whereas severe desaturation duration was 1 \( \pm \) 1 min, 21 \( \pm \) 15 s (range, 10 s to 4 min, 20 s).

All subjects recovered the \( \text{Sa}_2 \) fall within 5 min of the cessation of sputum induction. No differences among the three groups were found when comparing the fall in both \( \text{FEV}_1 \) and \( \text{Sa}_2 \) after saline solution inhalation and the duration of the overall sputum induction procedure mild-to-moderate desaturation, as well as severe desaturation. In addition, no correlation was found between \( \text{FEV}_1 \) and \( \text{Sa}_2 \) baseline values and the change in \( \text{Sa}_2 \) values among the three groups. In asthmatic subjects, no correlation was found between \( \text{PC}_{20} \) methacholine and the change in \( \text{Sa}_2 \).

**Discussion**

This study has shown a significant fall in \( \text{Sa}_2 \) during the stated sputum-induction procedure for subjects with bronchial asthma, subjects with a history of smoking, and healthy subjects. Additionally, the study has confirmed that sputum production induced by inhaling hypertonic saline solution is well tolerated in subjects with mild-to-moderate bronchial asthma, subjects with a history of smoking, and healthy subjects.

In accordance with the safety protocol proposed by Wong and Fahy,9 we pretreated the asthmatic subjects with inhaled salbutamol (200 \( \mu \)g) and regularly monitored pulmonary function during sputum induction. In asthmatic subjects with mild-to-moderate bronchial hyperreactivity, we found a mean maximal fall in \( \text{FEV}_1 \) of \(<2\%\) from the postbronchodilator value and no excessive bronchoconstriction. No subject developed sufficiently severe side effects to warrant premature interruption of the procedure. Several studies have reported the effects on spirometric results of sputum induction with hypertonic saline solution inhalation in asthmatic subjects.1,2,9,10,16–18 In those studies, subjects were pretreated with bronchodilators before sputum induction. However, pretreatment did not prevent excessive bronchoconstriction in all asthmatic subjects undergoing sputum induction,1,2,16 especially in those with low baseline \( \text{FEV}_1 \) values.9 In all cases, the hypertonic saline solution-induced falls in \( \text{FEV}_1 \) were easily and quickly reversed with an inhaled \( \beta_2 \)-agonist. Moreover, no subject developed refractory bronchoconstriction requiring hospitalization or treatment by an emergency department, even when sputum production was induced in patients with severe or uncontrolled asthma.10 In addition, other side effects, such as spontaneous cough1,10 and salty taste,1 were reported in asthmatic subjects. The effects on spirometric results of the hypertonic saline solution inhalation were caused by the bronchoconstrictive activity of nonosmotic solutions in subjects with bronchial hyperreactivity.19 At present, inhaled hypertonic saline solution is also used as a nonpharmacologic method of bronchial challenge both in children6 and adults7,8 with asthma.

In both smoking and healthy subjects, we induced sputum production by subject inhalation of hypertonic saline solution without pretreatment while we regularly monitored lung function. If we observed a fall in \( \text{FEV}_1 \) values of \( >20\% \) from the baseline value, we discontinued the saline solution nebulization and promptly administered 200 \( \mu \)g salbutamol (inhaled). Only 1 of 14 subjects who smoked experienced excessive bronchoconstriction (which rapidly reversed itself after salbutamol inhalation). This subject had a history of smoking of 60 pack-years.

It has been reported that smoking subjects generally tolerate sputum induction.3–5 To date, however, no study has addressed the safety of this technique in smokers. In one study,3 after inhalation of hypertonic saline solution, no fall in \( \text{FEV}_1 \) was detected in 12 healthy smokers pretreated with salbutamol. In another,4 excessive bronchoconstriction was observed in 2 of 46 smokers with chronic airway obstruction who were not treated before sputum induction. Thus, when considered in the light of published data,3,4 our results suggest that bronchodilator pretreatment can minimize the bronchoconstrictive effect of hypertonic saline solution in smoking subjects. In addition, although the mechanism of this bronchoconstriction is not well understood, the proinflammatory activity of hypertonic saline solution20 might play a role in this process.

We also observed a significant fall in \( \text{Sa}_2 \) during the inhalation of hypertonic saline solution in subjects with bronchial asthma, in subjects with a history of smoking, and in healthy subjects. To separate the effect of salbutamol from that of sputum induction on \( \text{Sa}_2 \) in asthmatic patients, we measured \( \text{Sa}_2 \) before and 15 min after salbutamol inhalation. We considered the latter measurement as the baseline \( \text{Sa}_2 \) for sputum induction. Assuming that the oxygen dissociation curve is normal and that the pH is 7.4, the fall in \( \text{Sa}_2 \) from 97.7 to 91.8% in asthmatic patients represents a fall in \( \text{Pa}_2 \) from 13.3 to 8.3 kPa; in smokers the fall from 97.5 to 92.9% represents a fall in \( \text{Pa}_2 \) from 13.3 to 8.8 kPa; and in healthy subjects the fall from 97.8 to 92.1%.

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represents a fall in $P_{aO_2}$ from 13.3 to 8.3 kPa.\textsuperscript{21} The maximal duration of severe desaturation was 4 min, 10 s in asthmatic subjects; 5 min, 30 s in subjects with a history of smoking; and 4 min, 20 s in healthy subjects. In addition, the fall in $S_{aO_2}$ recovered within the 5-min period after cessation of the procedure in asthmatic patients, smokers, and healthy subjects.

In this study, we provide the first evidence of a significant but transient fall in $S_{aO_2}$ due to the inhalation of hypertonic saline solution in asthmatic patients, smoking subjects, and healthy subjects. Recently, marked arterial desaturation was reported in HIV-positive patients during sputum production induced by hypertonic saline solution.\textsuperscript{11,12} In these studies, healthy subjects had a fall in $S_{aO_2}$ lower than that observed in HIV-positive patients. This drop is probably due to abnormalities of ventilation-perfusion ratios throughout the lung, which are probably caused by direct deposition of saline solution into peripheral airways.

In conclusion, our study confirmed that sputum induction is well tolerated in asthmatic patients, smokers, and healthy subjects. Moreover, we found that inhalation of saline solution did not induce significant effects on spirometric results in mild-to-moderate asthmatic patients pretreated with salbutamol, though it caused excessive bronchoconstriction in heavy smokers who had not been pretreated with salbutamol. Finally, we demonstrated that all the subjects had significant but transient and self-reversing oxygen desaturation. Although this effect was not related to baseline $S_{aO_2}$ in subjects with normal oximetric results, it leads us to recommend that in subjects who are hypoxic before sputum induction, $S_{aO_2}$ should be monitored during this procedure.

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REFERENCES

5 Stanesco D, Sanna A, Veriter C, et al. Airways obstruction, chronic expectoration, and rapid decline of $FEV_1$ in smokers are associated with increased levels of sputum neutrophils. Thorax 1996; 51:267–271
13 American Thoracic Society. Standards for diagnosis and care of patients with chronic obstructive pulmonary disease (COPD) and asthma. Am Rev Respir Dis 1987; 136:225–244