Uridine 5’-triphosphate (UTP) is a naturally occurring agonist for P2Y2 receptors on the apical surface of ciliated respiratory epithelium. UTP stimulates salt and water transport and cilia beat frequency in human airway epithelium in vitro. Single, inhaled doses of UTP stimulate mucociliary clearance in conscious, intubated sheep and in patients with mild chronic bronchitis (smokers and former smokers), suggesting that UTP may be useful for obtaining deep-lung sputum specimens suitable for diagnostic purposes.

Study objective: UTP is being developed for the cytologic diagnosis of lung cancer under the compound number INS316 Solution for Inhalation (Inspire Pharmaceuticals; Durham, NC). Its ability to improve the quality of expectorated sputum was tested in the current study.

Design: Placebo-controlled, double-blind, escalating two-period cross-over study.

Setting: Outpatient volunteers.

Patients or participants: Twenty-six patients with mild chronic bronchitis.

Intervention: Patients attempted to expectorate a specimen spontaneously, following a single inhaled dose of INS316 (10 to 180 mg), and following placebo.

Measurements and results: Sputum weight, sputum cell content, spirometry, and oxyhemoglobin saturation. Only 28% of these patients were able to expectorate a macrophage-containing, deep-lung specimen spontaneously or following inhalation of placebo. In contrast, 85% of the patients were able to produce a specimen following inhalation of INS316. The average weight of the sputum expectorated was increased fourfold by placebo and 10-fold by INS316. A mild transient decrease in pulmonary function was observed following INS316 administration.

Conclusion: A single dose of INS316 safely improves the ability of patients with mild chronic bronchitis to expectorate a deep-lung sputum specimen suitable for cytologic evaluation.

Key words: diagnostic tests; lung neoplasms; mucociliary clearance; purinergic P2; receptors; uridine triphosphate

Abbreviations: MMEF = maximal mid-expiratory flow rate; UTP = uridine 5’-triphosphate
e vivo in sheep. Recently, UTP has been shown to stimulate mucociliary clearance in patients with chronic bronchitis and cough clearance in patients with primary ciliary dyskinesia. This pharmacologic activity profile of UTP suggests that P2Y2 receptor agonists may have therapeutic potential in individuals in whom mucociliary clearance is impaired, such as in chronic bronchitis or cystic fibrosis. However, UTP is rapidly degraded enzymatically within sputum and by airway epithelial cells, and thus may not be suitable as a therapeutic agent in the treatment of these chronic diseases. Other enzymatically resistant, longer-acting P2Y2 agonists are being developed for these indications by Inspire Pharmaceuticals (Durham, NC). UTP, by virtue of its rapid onsets of action and rapid degradation, may be ideally suited as an agent for obtaining deep-lung sputum specimens suitable for diagnostic purposes. Inspire Pharmaceuticals is developing UTP solutions for inhalation for such indications under the compound number INS316.

Because lung tumors and other preneoplastic lung lesions shed cells that can be identified in sputum specimens, sputum cytology has become one component in the diagnosis of lung cancer. The reliability of the results of sputum cytology is highly dependent on the quality of the specimen obtained by the clinician. For the diagnosis of lung cancer, the ideal sputum specimen contains a large number of cells from deep within the lung because such specimens are more likely to contain exfoliated cells from lesions.

Many patients have difficulty spontaneously producing such evaluable sputum specimens, which has been recognized ever since the earliest attempts at developing screening protocols. Various methods and procedures have been employed over the years to enhance the quality and quantity of sputum expectorated for diagnostic purposes. Among these is inhalation of hypertonic saline solution or heated hypertonic saline solution at concentrations ranging from 3 to 15% sodium chloride, with or without added propylene glycol. The safety of this method has not been rigorously assessed but has been reported to cause significant acute decreases in pulmonary function. Another method is to have the subject collect multiple early-morning spontaneous expectorations at home or to pool these collections for a period of ≥3 days, which can involve complicated breathing and coughing maneuvers. Because these techniques are not reliable and are often unsuccessful, more invasive and costly procedures such as bronchoscopy are often performed to obtain evaluable specimens.

The development of INS316 as a diagnostic adjunct will require the testing of the compound in the appropriate subject population—patients confirmed to have the disease or subjects at high risk of acquiring the disease. Smokers and former smokers with mild chronic bronchitis, although not the population at highest risk of acquiring lung cancer, were selected as an appropriate population for the initial evaluation of INS316 as a sputum induction agent. The current study was designed to determine the safety, tolerability, and potential utility of single inhaled doses of aerosolized INS316 as an acute-use agent for conveniently obtaining deep-lung sputum specimens suitable for cytologic diagnostic purposes.

**Materials and Methods**

The study was approved by the Committee on the Protection of the Rights of Human Subjects of The University of North Carolina at Chapel Hill, and all patients gave their written informed consent. The study was conducted using the ethical standards of Good Clinical Practice of the Code of Federal Regulations, Title 21.

**Patients**

Twenty-six patients (12 men and 14 women; mean age, 43.4 years; range, 24 to 60 years), smokers and former smokers (mean, 36.9 pack-years; range, 5 to 160 pack-years), meeting the American Thoracic Society criteria for chronic bronchitis19 were enrolled in this study. All patients had FEV1 ≥65% predicted normal for age, height, and gender, and oxyhemoglobin saturation ≥92% on room air. Patients also demonstrated a <15% increase in FEV1 in response to inhaled bronchodilator. No patients had evidence of other significant illnesses, including pulmonary exacerbations, during the 4 weeks preceding enrollment. No patients had overproduction of mucus due to other causes such as bronchiectasis or infectious disease. Patients were excluded if they had changes in antimicrobial, bronchodilator, anti-inflammatory, or corticosteroid medications or had started taking new medications, including over-the-counter expectorants or cough suppressants, during the 2 weeks prior to enrollment into the study. Patients were also excluded if they had received an investigational drug or experimental therapy during the 30 days preceding enrollment.

**Study Protocol**

The trial was double blinded, placebo controlled, randomized, and cross-over in design. In an ascending-dose fashion, five successive cohorts were studied—each cohort consisting of five patients who received one nominal dose of INS316 during one treatment visit and placebo during a separate treatment visit. After the completion of each cohort, the next higher dose-level cohort was opened for enrollment if no significant adverse events occurred within the cohort. If adverse events did occur within a cohort, the safety data were reviewed in a blinded fashion by the investigator and an external medical safety consultant before proceeding further in the study. Following the completion of each dosing cohort, the institutional review board was informed of the nature of all adverse events and was informed of the basis for progressing to the next dose level.

Each patient attended an initial screening visit to assess the patient’s qualifications for enrollment, including response to a...
bronchodilator, and to determine other baseline values. The screening visit included spirometry and assessment of the response to 180 μg of albuterol (two 90-μg puffs from a metered-dose inhaler) to exclude patients with reversible airflow disease. Patients demonstrating a ≥ 15% increase in FEV1 following albuterol treatment were excluded from participating. Also at the screening visit, patients were asked to attempt to spontaneously expectorate a sputum specimen.

Following the screening visit, each patient attended two additional treatment visits separated by no less than 24 h and by no more than 7 days. During each of the treatment visits, patients received a single, inhaled dose (4 mL in the nebulizer) of either INS316 (10 mg, 20 mg, 60 mg, 100 mg, or 180 mg) or placebo (normal saline solution) by a PARI LC Plus jet nebulizer (PARI Respiratory Equipment; Monterey, CA) powered by a Pulmo-Aide compressor (Sunrise Medical; Somerset, PA). Each patient received active study drug on 1 of the 2 treatment days and placebo on the other treatment day, with the order being randomized and blinded to the patient and to all site personnel administering study drug and recording data. All solutions of INS316 were sterile, aqueous solutions with osmolality adjusted to 300 mOsm/L with sodium chloride as needed, pH adjusted to 7.2, and stored under refrigeration. Placebo was supplied as a sterile solution of 0.9% saline solution in single-use plastic vials stored at room temperature. All vials were stored in the central institutional pharmacy and dispensed as needed to the investigator in a blinded fashion.

Patients inhaled the dose using a standard breathing pattern of exhalation toward residual volume, slow (2- to 4-s) inhalation to total lung capacity, a 2- to 4-s breath-hold, and slow exhalation. A dose was considered completed after 15 min of inhalation or when nebulization ceased, whichever occurred first. Patients regularly receiving an oral or inhaled bronchodilator continued to take their medication as prescribed, but with the following limitations: (1) no bronchodilator drug was permitted within the 2-h period preceding administration of study drug; and (2) subsequent doses of bronchodilator were to be withheld until after completion of all postdose assessments.

Safety assessments, including spirometry, were performed within 1 h prior to dosing and again within 30 min after completion of study drug administration. Oxyhemoglobin saturation was monitored continuously by pulse oximetry from 1 h prior to dosing to 45 min after completion of study drug administration. Patients were discharged 2 h following study drug administration after final safety assessments had been performed, including oxyhemoglobin saturation and spirometry. Approximately 24 h after each treatment visit, the patients received a follow-up telephone call to monitor any adverse events.

Sputum Collection and Analyses

At the screening visit, patients were asked to cough and to expectorate any sputum that could be produced spontaneously into a preweighed specimen container. Expectorated sputum was also collected during the following periods in separate preweighed containers: from the beginning of dosing to 5 min immediately following completion of dosing, 5 to 30 min following completion of dosing, and 30 min following completion of dosing to the time of discharge from the study unit (approximate 2 h following completion of dosing). Sputum specimen weights were recorded, and the specimens were stored refrigerated until processed for cytology. Two slides were prepared for cytologic evaluation from each specimen by smearing a selected portion of the specimen between two microscope slides. The slides were air-dried: one slide was Papanicolau (Pap) stained, and the other slide was stained with the Wright-Giemsa stain (for identification of eosinophils). Slides were evaluated in a blinded fashion at × 400 magnification. The total number of macrophages, ciliated epithelial cells, eosinophils, and neutrophils were counted on 10 separate, nonoverlapping fields across the entire slide, and the data were recorded as the average number of cells per field, rounding to the nearest whole number.

Data Analysis

The study was conducted primarily as a phase 1 clinical trial to determine safe and tolerable doses of INS316 for future efficacy studies. The number of patients enrolled into each cohort was chosen to identify frequently occurring adverse events and was not designed to provide strong statistical power to efficacy measurements. Accordingly, the number of patients in each dosing cohort was relatively small and statistical comparisons between cohorts were not performed; rather, for statistical comparative analyses, all cohorts were combined. Results are presented as the mean ± SEM unless otherwise stated. Paired data from measurements made at different times for each patient, and unpaired data were compared using the Wilcoxon matched-pairs, signed-rank test or Student t test. Categorical data were compared using the McNemar test. Statistical significance was taken as p ≤ 0.05 in all comparative analyses.

One patient discontinued because of a respiratory infection (not attributed to treatment) following treatment with active study drug (100 mg) but before receiving placebo. This patient was replaced; consequently, the 100-mg cohort consisted of six patients. This patient was included in the analyses of all of the safety data. In the statistical analysis of the sputum data, the patient is included only in the analyses in which specimens obtained spontaneously at screening and following INS316 are compared.

RESULTS

Sputum Analyses

Figure 1 and Table 1 illustrate the ability of single inhaled doses of INS316 to enhance sputum expectoration in patients with mild chronic bronchitis. Only 27% of patients were able to spontaneously produce a deep-lung sputum specimen at the screening visit. In contrast, inhalation of INS316 significantly increased the number of patients who expectorated macrophage-containing sputum (85%) and increased the average amount of sputum expectorated 10-fold, whereas inhalation of placebo had little or no effect on sputum expectoration.

Figure 1, top, A shows the total amount of sputum collected for each specimen for each dosing cohort. Sputum production appeared to be related to dose level, with the 100-mg and 180-mg doses yielding the greatest quantity of sputum, though there are insufficient numbers of patients within these small cohorts to permit reliable statistical comparisons. Figure 1, bottom, B illustrates that the average weight of expectorated sputum was increased with INS316 (all doses combined) immediately following dosing (from the beginning of dosing to 5 min after dosing), whereas no effect was observed following treatment with placebo. The increase in sputum weight expec-
torated following treatment with INS316 was also observed during the 5- to 30-min postdose period, but no stimulatory effect was observed thereafter, consistent with the demonstrated short half-life of INS316.12

Many individuals were unable to produce a specimen within 5 min of dosing and needed up to 30 min to produce the specimen (individual patient data not shown). Because a single, combined specimen ordinarily would be collected in a clinical setting, the data from the sputum collected from the beginning of dosing through 30 min after dosing were evaluated in a combined fashion. Placebo administration resulted in only a twofold increase in the weight of sputum expectorated during this collection period compared to the spontaneous expectoration at screening. In contrast, INS316 produced an eightfold increase in the weight of sputum expectorated within 30 min after dosing compared to the spontaneous expectoration.

Figure 1. At the screening visit, patients were asked to expectorate a spontaneous sputum specimen in a preweighed container. Sputum specimens were also collected and weighed following each dosing in three separate preweighed containers corresponding to the following postdosing time periods: 0 to 5 min, 5 to 30 min, and 30 min to time of discharge (approximately 2 h). Top: A: the total sputum collected spontaneously and for each treatment period is shown within each dose level cohort. Bottom: B: The sputum collected during each time period is shown (all dose cohorts combined). Values are means ± SEM for all dosing cohorts combined. *p ≤ 0.05, significantly greater than spontaneous; †p ≤ 0.05, significantly greater than placebo.

The low average sputum weights obtained spontaneously and following treatment with placebo were largely due to the fact that many patients were unable to produce any sputum specimen. Table 1

Table 1—Patients Unable to Expectorate Sputum Containing Macrophages*

<table>
<thead>
<tr>
<th>Doses, mg</th>
<th>Spontaneous</th>
<th>Placebo</th>
<th>Active Drug</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>2/5</td>
<td>1/5</td>
<td>1/5</td>
</tr>
<tr>
<td>20</td>
<td>5/5</td>
<td>5/5</td>
<td>2/5</td>
</tr>
<tr>
<td>60</td>
<td>4/5</td>
<td>3/5</td>
<td>0/5</td>
</tr>
<tr>
<td>100</td>
<td>4/6</td>
<td>4/5</td>
<td>1/6</td>
</tr>
<tr>
<td>180</td>
<td>4/5</td>
<td>5/5</td>
<td>0/5</td>
</tr>
<tr>
<td>All doses</td>
<td>19/26</td>
<td>18/25</td>
<td>4/26†</td>
</tr>
</tbody>
</table>

*p Values are expressed as the no. of patients unable to expectorate sputum containing macrophages/total no. of patients in the cohort. \( \text{p} = 0.0001 \), significantly different from spontaneous by the McNemar test.

†p = 0.0005, significantly different from placebo by the McNemar test.
compares the number of patients unable to expectorate a deep-lung sputum specimen spontaneously at the screening visit with the number unable to expectorate within 30 min following treatment with placebo and with INS316. Nineteen of 26 patients were unable to expectorate sputum spontaneously at screening; similarly, 18 of the 25 patients were unable to expectorate sputum after treatment with placebo (two of the patients shown in Table 1 produced a small “specimen” spontaneously in which no macrophages could be identified). In contrast, following treatment with INS316, all but four patients were able to expectorate a sputum specimen within 30 min (all doses combined, \( p < 0.001 \)). Two of these four patients were unable to expectorate a sputum specimen during any of the visits.

Examination of individual patient data (not shown) indicated that 30% of the patients expectorated a specimen that contained no macrophages at some time during the study, and 7% of all the specimens collected in the study contained no macrophages. This amount is in addition to the 56% of all collections in which no specimen was obtained at all. To further assess the ability of INS316 to improve the quality of the specimen expectorated, transition tables (Table 2) were constructed to evaluate patients’ treatment-related change from being unable to expectorate macrophage-positive specimens.

It is recognized among cytologists that the quality of sputum expectorated is an important factor in obtaining accurate diagnoses. For this reason, sputum quality was also assessed by a semiquantitative analysis of the cell contents of the sputum. Figure 2 illustrates the effect of INS316 (all doses combined) on the cell contents of expectorated sputum. Treatment with INS316 increased the average sputum content of macrophages, ciliated epithelial cells, eosinophils, and neutrophils compared to placebo, which had no ability to increase these cell types in sputum. One exception to this was noted in the neutrophil content of sputum. A few patients had relatively high neutrophil counts in the spontaneous specimens, which was not observed in subsequent sputum specimens following placebo or INS316 in these same patients. Consequently, treatment with INS316 did not significantly increase the average neutrophil content relative to values from the spontaneous specimens, although INS316 did significantly increase the average neutrophil content of sputum relative to placebo.

Cell viability was not measured in the current study, although it is highly unlikely that INS316 adversely affects sputum cell viability. General cellular and nuclear morphology and cell integrity parameters were not adversely affected by INS316 administration in a previously reported study in patients with lung cancer.26 and as described in the introduction above, UTP has been demonstrated to stimulate, not impair, many functions of airway epithelial cells.

Safety and Tolerability

No serious adverse events were reported during the study. Table 3 lists the incidence of the most frequently reported adverse events (cough, throat irritation, wheezing, oxyhemoglobin desaturation, chest pain, dyspnea, etc.). Following treatment with placebo or with INS316, most adverse events occurred essentially concurrently shortly following administration. The events were generally mild and resolved spontaneously before discharge. The nature and time of onset of most of the frequently occurring events following INS316 administration are consistent with the drug producing a rapid mobilization of airway fluid. Accordingly, coughing was the most frequent symptom, occurring in 62% of patients following INS316 treatment compared to 24% following treatment with placebo. All of the coughing events were rated as mild, except for one event reported as severe in the 180-mg cohort; all coughing events produced sputum and resolved without treatment, typically within 10 to 30 min. A few patients reported increased coughing with sputum production for several hours following INS316 administration. One patient receiving the highest dose of INS316 experienced a set of events consistent with bronchospasm. These included oxyhemoglobin saturation decrease to < 85%, increased bilateral rhonchi, mild tachycardia, and mild diaphoresis, all of which were reversed after treatment with albuterol. In addition, one patient was discontinued from the study after acquiring a moderate respiratory infection on the day following the 100-mg dose of INS316 that was not attributed to treatment. No patients reported adverse events indicative of bronchospasm after the time of discharge during the follow-up telephone calls to the patients on the day after dosing.

| Table 2—Transition To/From Macrophage-Positive Sputum* |
|----------------|----------------|----------------|----------------|
|                | Placebo | Placebo | INS316 | INS316 |
| Findings        | Negative | Positive | Negative | Positive |
| Spontaneous negative | 15      | 3       | 4       | 15     |
| Spontaneous positive | 3       | 4       | 0       | 7      |

*Sputum specimens were designated as either positive or negative, indicating that the specimen either contained macrophages or did not contain macrophages, respectively. One patient withdrew after treatment with INS316 prior to treatment with placebo. INS316 vs screen probability ≤ 0.0001 by the McNemar test.
Spirometry and oxyhemoglobin saturation were assessed as safety indicators shortly before treatment, shortly following treatment, and at the time the patient was discharged from the study unit. Table 4 presents a summary of the spirometry and oximetry data. A decrease in FEV₁ was detected shortly following administration with INS316 (mean, −8.6%). The greatest changes in FEV₁ occurred at the higher doses (mean change, −0.42 L with 60 mg, −0.22 L with 100 mg, and −0.41 L with 180 mg), with smaller changes occurring following the 10-mg and 20-mg doses (mean changes, −0.10 L and −0.012 L, respectively). At the time of discharge, the average FEV₁ had increased but remained slightly lower than predose values (mean, −2.91%). A small average decrease in FVC was detected shortly following administration with INS316 compared to predose values (mean, −5.1%), with no detectable relation to the level of the dose (data not shown). The average FVC approximated predose values but was slightly lower at the time of discharge (mean, −1.3%). Maximal mid-expiratory flow rate (MMEF) values declined shortly following treatment with INS316 (mean, −15%) with no detectable relation to the level of the dose (data not shown). At the time of discharge, the average MMEF had increased but remained slightly lower than predose values (mean, −4.8%). A very small average decrease in oxyhemoglobin saturation was detected when measured shortly following dos-

![Graphs showing spirometry and oximetry data](image)

Figure 2. Sputum specimens were collected as described in Figure 1 and stored refrigerated until processed for cytology. Two direct smear slides were prepared from each specimen. One slide was stained with Papanicolaou for counting macrophages, ciliated epithelial cells, and neutrophils. The other slide was stained with Wright-Giemsa for counting eosinophils. Each cell type was counted on 10 separate, nonoverlapping fields across the entire slide, and the data were recorded as the average number of cells per field, rounding to the nearest whole number. Values are mean ± SEM of the number of cells counted per high-power field (hpf) [× 400]. *p ≤ 0.05, significantly greater than spontaneous; †p ≤ 0.05, significantly greater than placebo.

### Table 3—Most Frequent Adverse Events*

<table>
<thead>
<tr>
<th>Variables</th>
<th>Placebo</th>
<th>10 mg</th>
<th>20 mg</th>
<th>60 mg</th>
<th>100 mg</th>
<th>180 mg</th>
<th>All INS316 Doses</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients at risk</td>
<td>25</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>26</td>
</tr>
<tr>
<td>Cough</td>
<td>6</td>
<td>3</td>
<td>2</td>
<td>4</td>
<td>5</td>
<td>3</td>
<td>17</td>
</tr>
<tr>
<td>Throat tickle/irritation</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>3</td>
<td>8</td>
</tr>
<tr>
<td>Wheezing</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>3</td>
<td>0</td>
<td>2</td>
<td>7</td>
</tr>
<tr>
<td>O₂ saturation decrease</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>3</td>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td>Chest tightness/pain</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td>Dyspnea</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Sputum increased†</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>3</td>
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<tr>
<td>Dry mouth</td>
<td>5</td>
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<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>3</td>
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<tr>
<td>Dry throat</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>3</td>
</tr>
</tbody>
</table>

*Data are presented as No.
†Reported as a complaint by study patients.
FEV₁, L  3.09 (0.16)  3.10 (0.16)  3.07 (0.16)  3.11 (0.15)  2.86 (0.15)
FVC, L        4.08 (0.22)  4.06 (0.22)  4.04 (0.21)  4.06 (0.21)  3.87 (0.21)
MMEF, L/s     2.73 (0.25)  2.76 (0.06)  2.69 (0.23)  2.80 (0.21)  2.38 (0.20)
O₂ saturation, % 96.2 (0.39)  96.6 (0.34)  97.3 (0.30)  98.3 (0.29)  97.0 (0.29)

<table>
<thead>
<tr>
<th>Variables</th>
<th>Placebo</th>
<th></th>
<th></th>
<th>All INS316 Doses</th>
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<tbody>
<tr>
<td></td>
<td>Predose</td>
<td>30–45 min Postdose</td>
<td>At Discharge (Approx 2 h Postdose)</td>
<td>Predose</td>
<td>30–45 min Postdose</td>
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<td>3.09 (0.16)</td>
<td>3.10 (0.16)</td>
<td>3.07 (0.16)</td>
<td>3.11 (0.15)</td>
<td>2.86 (0.15)</td>
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<td>2.76 (0.06)</td>
<td>2.69 (0.23)</td>
<td>2.80 (0.21)</td>
<td>2.38 (0.20)</td>
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<tr>
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<td>96.2 (0.39)</td>
<td>96.6 (0.34)</td>
<td>97.3 (0.30)</td>
<td>98.3 (0.29)</td>
<td>97.0 (0.29)</td>
</tr>
</tbody>
</table>

*Values are means (SEM). Approx = approximately.
†p < 0.05 compared to predose values by Wilcoxon matched-pairs, signed-rank test.
‡p < 0.01 compared to predose values by Wilcoxon matched-pairs, signed-rank test.
§p < 0.001 compared to predose values by Wilcoxon matched-pairs, signed-rank test.
¶p < 0.0001 compared to predose values by Wilcoxon matched-pairs, signed-rank test.

Discussion

Obtaining a sputum specimen for diagnostic purposes is often difficult, even in patients with chronic bronchitis, because many patients simply are unable to produce an adequate, deep-lung specimen on request. The data from the current study suggest that a single, inhaled, small-volume dose of INS316 will significantly improve the success rate for obtaining sputum in these patients (Tables 1, 2). This improvement can be attributed to the pharmacologic activity of the drug, because an equal volume of normal saline solution was unable to improve sputum expectoration. Similarly, sputum cellularity was increased following administration of INS316, whereas normal saline solution had no noticeable effect (Fig 2).

Although coughing could conceivably contribute to enhanced sputum expectoration, such a nonspecific tussive effect is likely to be small. As was observed in the current study, many patients are unable to expectorate a specimen even when instructed to forcibly and repeatedly cough. The enhanced sputum production and cellularity following INS316 administration is more likely due to the immediate stimulatory effect of the compound on mucociliary clearance recently demonstrated by Bennett et al,6 who used gamma scintigraphy to observe and quantify the clearance of inhaled radio-labeled particles in a similar population of patients with mild chronic bronchitis. At baseline, only approximately 12% of the inhaled particles were cleared by a rapid-clearance mechanism. After this initial, rapid phase of clearance, only approximately one half of the remaining particles were cleared during the next 24 h. A single dose of UTP (INS316) increased the rate of this initial clearance phase approximately twofold compared to the no-treatment baseline clearance rate and approximately 50% over the clearance rate following placebo (normal saline solution) administration. More importantly, UTP increased the total amount of material cleared during the initial phase approximately threefold compared to the no-treatment baseline amount and approximately 50% over the amount cleared following placebo administration. These data suggest that the sputum came from a significantly greater portion of the lung following dosing with UTP in the study by Bennett et al and in the current study.

The ability of INS316 to improve sputum cellularity was observed at doses as low as 20 mg, which is consistent with the results of Bennett et al,6 in which a low dose of UTP (20 mg) increased the rate of mucociliary clearance to a similar degree as did a high dose of UTP (100 mg). These data collectively suggest that even the lowest doses of INS316 tested are at least acutely effective at stimulating the movement of airway material from the periphery of the lung into the upper airways and into the oropharynx. Because the number of patients in the current study was small and because the cell counting method was only semiquantitative, the data from the current study are probably not sufficient to conclude that the lowest doses of INS316 tested are as efficacious as higher doses.

INS316 was generally well tolerated in these patients. Adverse events were mostly confined to the respiratory system and were consistent with the rapid mobilization of airway secretions by INS316.
Accordingly, most patients exhibited coughing following INS316 administration, whereas only approximately 25% of the patients had significant coughing following administration of normal saline solution. A small number of patients experienced mild symptoms of throat irritation, wheezing, and chest tightness, which generally were short-lived and resolved spontaneously. The transient decreases in lung function following INS316 administration (Table 4) are also consistent with the pharmacologic activity of the drug to promote fluid movement into the airways. These effects were not consistently observed with placebo and thus could not be attributed simply to the volume of fluid delivered by the nebulizer.

Similar transient decreases in oxyhemoglobin saturation and FEV₁ immediately following sputum induction with hypertonic saline solution have been reported in asthmatics (FEV₁ decrease, 1.36 ± 5.6% [mean ± SD]), smokers (FEV₁ decrease, 7.6 ± 11.8%), and normal, healthy individuals (FEV₁ decrease, 0.05 ± 9.6%). Although the decrements in lung function following dosing with INS316 were mild to moderate in the current study, it is not known if patients with more severe pulmonary disease, who are likely to be evaluated for lung cancer, will respond similarly to INS316 dosing. Future studies of INS316 in patients with significantly compromised lung function will require careful assessment of pulmonary function following dosing.

It should be noted that one can expect a small number of patients to exhibit evidence of airway hyperreactivity following administration of high doses of INS316. One patient in the current study who received the 180-mg dose required the use of a bronchodilator following dosing. In previous clinical trials of INS316, two patients with cystic fibrosis and one other patient with chronic bronchitis, all receiving the 180-mg dose, required administration of albuterol following dosing. In each of these cases, the symptoms of bronchoconstriction were immediately relieved after albuterol administration. Because adverse events and a higher incidence rate of bronchoconstriction have been observed at the 180-mg dose of INS316, future studies of INS316 will evaluate doses only as high as 100 mg. One should also note that the incidence of bronchoconstriction with the highest dose of INS316 is similar to that reported by others when hypertonic (3%) saline solution was used to induce sputum in smokers.19

Since this article was initially submitted, Tamaoki et al27 reported the results of a study of sputum induction comparing UTP with 3% hypertonic saline solution in healthy individuals and in patients with mild-to-moderate asthma. These investigators demonstrated in both populations improved sputum production with similar cellularity and better postinduction pulmonary function following sputum induction with UTP compared to hypertonic saline solution. In addition, nausea and vomiting were commonly reported following administration of hypertonic saline solution, whereas these side effects were not observed with UTP induction.

The ability of INS316 to reliably induce highly cellular sputum specimens may increase the utility of sputum for cytologic diagnosis of lung cancer. Sputum cytology is regarded as inexpensive, simple, and safe; however, the sensitivity of sputum cytology as a means of diagnosing lung cancer has not been sufficiently high to warrant widespread screening. Part of the lack of sensitivity of sputum cytology derives from the difficulty in obtaining high quality sputum specimens from the subject. Such was also our finding in the current study in which approximately 75% of patients with chronic bronchitis were unable to spontaneously produce a sputum specimen on request. Furthermore, considering those patients who were able to spontaneously expectorate a sputum specimen, one half of these patients were unable to produce a specimen following inhalation of normal saline solution (Table 2), even though normal saline solution might be expected to enhance expectoration. Thus, a patient’s ability to spontaneously expectorate an evaluable sputum specimen appears to be unpredictable within this population. This problem is especially characteristic of former smokers, who comprise approximately 40% of the new cases of lung cancer detected annually in the United States. The data from the current study suggest that INS316 may be a useful, well-tolerated tool for acquiring high-quality sputum specimens containing cells of diagnostic interest, especially in cases in which the subject is unable to produce a specimen spontaneously.

**References**

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