

Safety and Tolerability of Denufosal Tetrasodium Inhalation Solution, a Novel P2Y₂ Receptor Agonist: Results of a Phase 1/Phase 2 Multicenter Study in Mild to Moderate Cystic Fibrosis

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Summary. Denufosal tetrasodium (INS37217) is a selective P2Y₂ agonist that stimulates ciliary beat frequency and Cl⁻ secretion in normal and cystic fibrosis (CF) airway epithelia, and is being investigated as an inhaled treatment for CF. The Cl⁻ secretory response is mediated via a non-CFTR pathway, and the driving force for Cl⁻ secretion is enhanced by the effect of P2Y₂ activation to also inhibit epithelial Na⁺ transport. Denufosal is metabolically more stable and better tolerated, and may enhance mucociliary clearance for a longer period of time than previously investigated P2Y₂ agonists. The goal of this phase 1/phase 2 study was to assess the safety and tolerability of single and repeated doses of aerosolized denufosal in subjects with CF. The study was a double-blind, placebo-controlled, multicenter comparison of ascending single doses of denufosal (10, 20, 40, and 60 mg, administered by inhalation via the Pari LC Star[®] nebulizer) vs. placebo (normal saline), followed by a comparison of twice-daily administration of the maximum tolerated dose (MTD) of denufosal or placebo for 5 days. Thirty-seven adult (18 years of age or older) and 24 pediatric (5–17 years of age) subjects with CF were evaluated in five cohorts. Subjects were randomized in a 3:1 ratio to receive either denufosal or placebo within each cohort. The percent of subjects experiencing adverse events was similar between the denufosal and placebo groups. The most common adverse event in subjects receiving denufosal was chest tightness in adult subjects (39%) and cough in pediatric subjects (56%). Three (7%) subjects receiving denufosal and one (7%) subject receiving placebo experienced a serious adverse event. Forced expiratory volume in 1 sec (FEV₁) profiles following dosing were similar across treatment groups, with some acute, reversible decline seen in both groups, most notably in subjects with lower lung function at baseline. In conclusion, doses up to 60 mg of denufosal inhalation

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solution were well-tolerated in most subjects. Some intolerance was noted among subjects with lower baseline lung function. Based on the results of this phase 1/phase 2 study, the Therapeutics Development Network (TDN) of the Cystic Fibrosis Foundation (CFF) and Inspire Pharmaceuticals, Inc., recently completed a multicenter, 28-day, phase 2 safety and efficacy clinical trial of denufosal inhalation solution in CF subjects with mild lung disease. **Pediatr Pulmonol.** 2005; 39:339–348. © 2005 Wiley-Liss, Inc.

Key words: INS37217; inhalation solution; denufosal tetrasodium; cystic fibrosis; FEV₁; lung function.

INTRODUCTION

Cystic fibrosis is a recessive genetic disease¹ which affects approximately 30,000 Americans.² Most deaths of patients with CF occur as a consequence of pulmonary disease. The disease is caused by mutations in the cystic fibrosis transmembrane regulator (CFTR) gene, which encodes for an apical membrane epithelial protein that functions as a cyclic AMP-regulated chloride channel and a regulator for other channels.³ Defective CFTR results in abnormal ion transport and airway surface liquid volume, producing reduced mucociliary and cough clearance and chronic infection of the respiratory tract.⁴ Although survival has increased with the advent of better treatments, the median age for survival is still only 32 years,² and patients have significant morbidity, including hospitalizations, during their lives.⁵

Additional therapeutic approaches are needed for the prevention and treatment of CF lung disease. In particular, agents that correct the underlying ion transport defects in the airways may prove useful in normalizing airway secretions, leading to improved mucociliary clearance, and prevention of chronic lung infections and progressive lung damage.

Nucleotide P2Y₂ agonists, such as uridine 5'-triphosphate (UTP) and diquafosol tetrasodium [P¹, P⁴-di(uridine 5') tetrphosphate, tetrasodium salt] (INS365), regulate certain activities of the human airway epithelium. The P2Y₂ receptor is abundant on the luminal surface of polarized epithelial cells, especially those lining mucosal surfaces exposed to the external environment.⁶ The effects of P2Y₂ agonists on epithelial cell function are numerous and include: stimulation of serosal to mucosal chloride and fluid transport by both inhibiting Na⁺ absorption and stimulating Cl⁻ secretion;^{7–11} enhancement of mucin secretion from goblet cells and submucosal glands, which promotes the trapping of foreign particles;^{12,13} increase in cilia beat frequency;¹⁴ and promotion of surfactant release from type II alveolar cells.¹⁵ Additionally, tracheal mucus velocity (TMV), a measure of mucociliary clearance in a single large airway, revealed the mucokinetic effects of nucleotides and various other agents in the lung in vivo.¹⁶ Denufosal tetrasodium [P¹-(uridine 5')-P⁴-(2'-deoxycytidine 5') tetrphosphate, tetrasodium salt] (INS37217), a new P2Y₂ dinucleotide analog of UTP, is being developed in clinical trial studies as a treatment for patients with CF.

Denufosal (INS37217) is a novel P2Y₂ agonist, and the pharmacological profile of denufosal parallels that of UTP and diquafosol (INS365),⁶ agonists which were previously tested in normal subjects and in subjects with CF.^{17–19} UTP was previously shown to activate Cl⁻ secretion and enhance MCC in normal subjects and patients with obstructive disease.^{20–22} Denufosal is metabolically more stable than previously tested P2Y₂ agonists,⁶ and may have longer-lasting effects in the airways of CF patients. Diquafosol (INS365) was shown to be approximately 10 times more stable than UTP on the mucosal surface of human nasal epithelial cells, and denufosal (INS37217) was shown to be approximately 50 times and 6 times more stable than UTP and diquafosol (INS365), respectively, on the mucosal surface of human nasal epithelial cells.⁶ Additionally, diquafosol (INS365) and denufosal (INS37217) were shown to have half-lives of about 3 hr and 25 hr, respectively, in CF sputum samples obtained from CF patients.⁶ The enhanced duration of action of denufosal (INS37217) and its ability to resist metabolism on the airway surface may allow for prolonged activation of the alternate chloride channel, and could provide a more effective treatment of CF lung disease. An earlier phase 1 study showed that denufosal was well-tolerated at doses up to 80 mg. Doses of 200 mg and above in nonsmokers (N = 40) and 100 mg and above in smokers (N = 40) were associated with mild cough, which was typically productive.²³

The primary objectives of this current study were to assess the safety and tolerability of single ascending doses of denufosal and repeated doses of the maximum tolerated dose of denufosal (INS37217) compared to placebo in subjects with CF. Secondary objectives were to obtain preliminary evidence of the pharmacological activity of denufosal (INS37217), as indexed by the amount of sputum expectoration over time and by a symptom questionnaire that assessed cough and sputum production.

MATERIALS AND METHODS

Study Design

The study was designed as a double-blind, randomized, multicenter comparison of ascending single inhaled doses of denufosal vs. placebo, followed by a comparison of twice-daily administration of the maximum tolerated dose (MTD) of denufosal or placebo. Adult and pediatric sub-

TABLE 1—Dosing for Adult and Pediatric Cohorts¹

	Part I (single dose in mg or placebo)			Part II (BID dosing)	Posttreatment
	1 ²	2 ²	3	4–8	9
Day					
Cohort 1A: Adult	10	20	40	MTD	Follow-up
Cohort 2A: Adult	20	40	60	MTD	Follow-up
Cohort 1B: Pediatric	10	20	40	MTD	Follow-up
Cohort 2B: Pediatric	20	40	60	MTD	Follow-up
Cohort 3A: Adult	20	40	60	MTD	Follow-up

¹MTD, maximum tolerated dose from part I.

²Escalation to next dose level was based on tolerability.

jects with CF were randomized in a 3:1 fashion to either receive denufosal (INS37217) or placebo within each of five cohorts (three adult cohorts and two pediatric cohorts), as shown in Table 1.

The first cohort was conducted in adults and included administration of denufosal in doses of 10, 20, and 40 mg. Upon completion of this and each subsequent adult cohort, the CFF Data Monitoring Committee (DMC) reviewed unblinded safety data in order to make recommendations concerning dose escalation for the next adult cohort. To ensure safety in younger subjects, the DMC reviewed data from the two completed adult cohorts prior to enrolling patients in the first pediatric cohort. The DMC then reviewed data from the two completed adult cohorts and the first pediatric cohort prior to enrolling patients in the second pediatric cohort. Due to an unanticipated imbalance between treatment groups in the second adult cohort with respect to baseline lung function that resulted in denufosal subjects having higher lung function at baseline, a change was made to the inclusion criteria for the third adult cohort. Subjects in this cohort were required to have a forced expiratory volume in 1 sec (FEV₁) 40–70% of predicted in order to obtain additional safety and tolerability data in subjects with compromised lung function. As the third adult cohort had compromised lung function, the evaluated doses were also changed to 20, 40, and 60 mg (intended doses were 40, 60, and 80 mg), and a 6-hr postdose spirometry measurement was added. Finally, the third pediatric cohort was dropped, as further escalation of doses beyond 60 mg was not necessary, given that the highest dose tested in adults was 60 mg.

As shown in Table 1, the treatment phase consisted of two parts conducted in the clinic. During part I (days 1–3), subjects received a series of up to three single ascending doses of denufosal or three single doses of placebo (the same volume of 0.9% w/v sterile sodium chloride solution) in an effort to establish an MTD. Part II consisted of twice-daily administration (approximately 10–12 hr between doses) of the subject's MTD for 5 consecutive days (days 4–8), starting 1–5 days after the completion of part I. Posttreatment follow-up procedures were conducted the morning of day 9, or the day after the subject was withdrawn for those subjects stopping early. In addition,

telephone follow-up was conducted 6–10 days post-treatment to query the subject about adverse experiences.

Clinical dose adjustment criteria were established for part I of the study in order to define individual subject MTDs and to ensure subject safety. At any visit during part I, subjects who experienced a $\geq 20\%$ postdose decline in FEV₁ compared with the predose FEV₁, or who had a sustained (>10 min) decline in oxyhemoglobin saturation to <85%, or a >8% sustained absolute decline from baseline, were considered to have exceeded their MTD and had to revert back to the previous dose level defined for their cohort. Additionally, the protocol allowed an investigator to lower a subject's MTD, if deemed necessary. Subjects were withdrawn from the study if they experienced a $\geq 20\%$ decline in FEV₁ from baseline at any predose measurement, a sustained decline in oxyhemoglobin saturation, a postdose decline in FEV₁ of $\geq 20\%$ from the predose value on any day during part II, or a serious adverse event attributed to the study medication.

The study was carried out using the Cystic Fibrosis Foundation Therapeutics Development Network (CFF TDN), with subjects recruited through their individual CF centers: 6 in network and 2 out of network centers were utilized. The TDN Coordinating Center Data Management Unit generated and maintained the randomization schedule for the study. Subjects were randomized in a 3:1 ratio to denufosal or placebo. Because randomization was not stratified by center due to expected small numbers of patients enrolled at some centers, an adaptive randomization procedure was put into place to ensure that at least one subject in any center enrolling two or more patients was assigned to placebo. Study personnel at clinical centers, study subjects, and all sponsor personnel remained blind to treatment assignments through the completion of the first four cohorts (adult and pediatric). Selected team members at the TDN Coordinating Center and the sponsor were unblinded prior to beginning the third adult cohort, in order to assess the need for that cohort. Modifications to the entry criteria were made at that time to ensure that sufficient numbers of patients with low lung function were included in that cohort. Study-site pharmacists were unblinded for the purpose of dispensing study drug.

Study Subjects

Subjects (adult subjects aged ≥ 18 years; pediatric subjects aged 5–17 years) were eligible if they had a confirmed diagnosis of CF by sweat chloride and/or genotype, an FEV₁ $\geq 50\%$ of predicted normal (cohorts 1 and 2), an FEV₁ 40–70% of predicted normal (cohort 3), and an oxyhemoglobin saturation of $\geq 90\%$ on room air. Subjects were required to be clinically stable, able to reproducibly perform spirometry maneuvers (in accordance with 1987 American Thoracic Society recommendations²⁴), and have no evidence of acute respiratory tract infection or current pulmonary exacerbation.

Key exclusion criteria included abnormal renal or liver function (serum creatinine ≥ 2 mg/dl or liver function tests (LFTs) $\geq 3 \times$ upper limit of normal), pregnancy or breastfeeding, electrocardiogram (ECG) changes consistent with clinically significant cardiovascular disease, abnormal chest x-ray other than CF, and changes in physiotherapy technique or antimicrobial, bronchodilator, anti-inflammatory, or corticosteroid medications during the week prior to screening. In addition, subjects taking inhaled cromolyn, hypertonic saline, intravenous or aerosolized antibiotics, antitussives, expectorants, or mucolytic agents were excluded. Medications routinely used in the management of CF were permitted during the study, including antimicrobial, anti-inflammatory, corticosteroid, and Pulmozyme[®] medications. All subjects or their guardians provided written informed consent, and the study was approved by the institutional review board at each of the eight clinical sites involved in the study.

Study Drug

Study drug was supplied by Inspire Pharmaceuticals, Inc., as a solution for inhalation. Denufosol was a sterile solution packaged in blow/fill/seal vials, each containing approximately 4.2 ml of solution, allowing delivery of 4 ml to the nebulizer cup for nebulization (Pari LC Star[®]). The placebo solution was 0.9% w/v solution of sterile sodium chloride, and the pH of the solution was within the range of 4.5–7.0. Denufosol and placebo were indistinguishable in appearance, smell, taste, and packaging, to maintain the blind. The concentrations of denufosol used in this trial were 2.5, 5, 10, and 15 mg/ml, which correlate to nebulizer doses of 10, 20, 40, and 60 mg denufosol, respectively. Each dose was considered complete after 15 min of inhalation or when nebulization stopped, whichever occurred first. All subjects were instructed to inhale doses using normal tidal breathing.

Safety Measures and Measures of Pharmacological Activity

The primary objective of the study was the evaluation of safety and tolerability. Safety measures included adverse events, pulmonary function tests (PFTs), oxyhemoglobin

saturation, vital signs, physical examination changes, clinical laboratory tests, 12-lead ECGs, rescue bronchodilator use, and rate of withdrawal from the study. Outcome measures for the assessment of pharmacological activity included expectorated sputum weight and responses to a respiratory symptom questionnaire.

All safety measures were assessed at the screening and follow-up visits. PFTs (FEV₁, forced vital capacity (FVC), and forced expiratory flow at 25%–75% of maximal lung volume (FEF_{25–75})), oxyhemoglobin saturation, and vital signs were also assessed predose, 15 min postdose, and 2 hr postdose on days 1, 2, 3, 4, and 8 (vital signs were also measured 30 and 60 min postdose). Pulmonary function testing was conducted in accordance with current American Thoracic Society recommendations.²⁴ Adverse events were continually assessed and recorded in the case report form by the principal investigator, subinvestigator, or research coordinator each day during the study.

On days 1, 4, and 8, expectorated sputum was collected (in preweighed, prelabeled containers) over two time periods, from 60 min predose until treatment with study medication, and for 75 min following the start of treatment (morning dose only). All sputum expectorated during each time period was collected, including sputum expectorated during a maneuver. Subjects were asked to perform the breathing and coughing maneuver at least once during the collection of sputum specimens. The coughing maneuver involved inhaling deeply three times, followed by a deep cough that would elicit expectoration of sputum into a preweighed specimen cup.

The symptom questionnaire was based on a section of a validated quality-of-life questionnaire, the CF Questionnaire (CFQ).²⁵ The section of the CFQ relevant to respiratory symptoms, such as cough and sinus congestion, was chosen as an exploratory parameter to be used for further development in later trials. Two questions that were expected to be affected by the pharmacologic activity of denufosol were modified to differentiate between types of congestion (sinus congestion vs. lung congestion) and cough (productive cough vs. nonproductive cough). The questionnaire was completed at screening, follow-up, and on each day of part II prior to any study procedures, including the administration of study medication.

Statistical Analysis

Data from all eligible subjects who received at least one dose of the study drug were included in analyses of safety and pharmacological activity. Adult and pediatric data were analyzed separately, and data from placebo subjects were pooled across dose cohorts to form separate adult and pediatric placebo groups for comparison. Due to the two-part study design, subjects on denufosol did not receive the same dose throughout the entire study (varied doses for part I, but consistent doses for part II). Therefore, comparisons were made between treatment groups (i.e., combined denufosol

subjects vs. placebo subjects) and/or dose groups (i.e., placebo vs. 10 mg, 20 mg, 40 mg, or 60 mg), as appropriate. Adverse events were attributed to the last dose the subject received before experiencing the event.

Baseline was defined as the measurement prior to and closest to the first administration of study medication. Testing for differences in baseline characteristics between treatment groups was performed using the Wilcoxon rank sum test. All data were summarized using descriptive statistics. At each visit and timepoint, testing for differences in pre- to postdose changes in FEV₁ (liters), oxyhemoglobin saturation, and sputum weight between each denofosol dose group and placebo was performed using the Wilcoxon rank sum test.

Since this study was not designed for formal hypothesis-testing of treatment group differences, any examination of trends among the various doses and placebo was exploratory in nature. All statistical tests were two-sided, and significance was assessed at the $\alpha=0.05$ level. No adjustments for multiple comparisons were made. All results are presented as means \pm standard deviation, unless otherwise specified.

RESULTS

Forty-six adult and 25 pediatric subjects were screened between June 26, 2001–July 29, 2002. Of the 46 screened

adult subjects, 38 were eligible and received at least one dose of study drug (9 placebo and 29 denofosol subjects). One subject inadvertently participated in the study twice; the subject’s second set of data is not included in any results. Nine (100%) adult subjects receiving placebo and 24 (86%) adult subjects receiving denofosol completed the study. Of the 25 screened pediatric subjects, 24 (6 placebo and 18 denofosol subjects) were eligible and received at least one dose of study drug. Five (83%) pediatric subjects receiving placebo and 17 (94%) pediatric subjects receiving denofosol completed the study. Reasons for discontinuation included patient decision, physician decision, meeting the protocol-defined safety and spirometry stopping criteria, and adverse events as assessed by the investigator (Table 2A).

Overall, treatment groups were similar with respect to most demographic and baseline clinical characteristics in both adult and pediatric subjects (Table 2), with the exception that subjects receiving denofosol had a higher FEV₁ of percent predicted on average than subjects receiving placebo. The majority of the difference in the adult treatment group means occurred in cohort 2A, where subjects in the denofosol group had a considerably higher mean FEV₁ percent of predicted (96.3 ± 13.3) as compared to the placebo group (68.0 ± 16.2), although the difference was not statistically significant ($P = 0.056$).

TABLE 2A—Demographic and Baseline Clinical Characteristics of Treatment Groups

Characteristic	Adult subjects		Pediatric subjects	
	Denofosol (N = 28)	Placebo (N = 9)	Denofosol (N = 18)	Placebo (N = 6)
Screened	46		25	
Randomized and dosed	29 ¹	9	18	6
Completed	24	9	17	5
Discontinued	5	0	1	1
Adverse event	1	0	0	0
Met stopping criteria	2	0	1	0
Patient decision	1	0	0	1
Physician decision	1	0	0	0
Mean age \pm SD, years	22.5 (4.3)	24.6 (6.5)	12.1 (3.8)	13.2 (3.2)
Male, n (%)	15 (54)	5 (56)	12 (67)	3 (50)
Caucasian, n (%)	28 (100)	9 (100)	18 (100)	6 (100)
CF genotype, n (%)				
Δ F508 homozygous	20 (71)	5 (56)	11 (61)	3 (50)
Δ F508 heterozygous	6 (21)	4 (44)	6 (33)	3 (50)
Other	2 (7)	0	1 (6)	0
Rescue bronchodilator use, n (%)				
\geq 3 times/week	5 (18)	2 (22)	1 (6)	0
<3 times/week	12 (43)	4 (44)	7 (39)	4 (67)
Does not use	11 (39)	3 (33)	10 (56)	2 (33)
Mean FEV ₁ % predicted \pm SD	76.1 (23.7)	64.6 (16.9)	90.8 (16.7)	80.1 (21.1)
Mean height \pm SD (cm)	169.8 (8.8)	172.8 (9.9)	148.2 (20.3)	153.6 (15.0)
Mean weight \pm SD (kg)	62.4 (10.0)	61.4 (13.9)	41.5 (17.2)	47.7 (13.0)
Mean oximetry \pm SD (%)	96.4 (2.4)	95.9 (2.3)	97.2 (1.4)	96.0 (1.7)

¹One subject was inadvertently randomized and dosed in error, as this patient had already been enrolled in study previously at a different site. Subject’s second set of data is not included in any results.

TABLE 2B—Demographic and Baseline Clinical Characteristics of Treatment Groups by Cohort

Characteristic	Adult subjects					
	Cohort 1A		Cohort 2A		Cohort 3A	
	Denufosal (N = 9)	Placebo (N = 3)	Denufosal (N = 10)	Placebo (N = 3)	Denufosal (N = 9)	Placebo (N = 3)
Mean age ± SD, years	20.7 (2.7)	26.7 (11.6)	23.1 (2.6)	21.7 (2.9)	23.7 (6.4)	25.3 (2.5)
Male, n (%)	5 (56)	2 (67)	5 (50)	1 (33)	5 (56)	2 (67)
Mean FEV ₁ % predicted ± SD	78.8 (19.9)	74.5 (18.2)	96.3 (13.3)	68.0 (16.2)	50.9 (7.5)	51.2 (10.9)
Characteristic	Pediatric subjects					
	Cohort 1B		Cohort 2B			
	Denufosal (N = 9)	Placebo (N = 3)	Denufosal (N = 9)	Placebo (N = 3)		
Mean age ± SD, years	12.2 (3.8)	14.7 (2.5)	11.9 (3.9)	11.7 (3.5)		
Male, n (%)	6 (67)	1 (33)	6 (67)	2 (67)		
Mean FEV ₁ % predicted ± SD	94.6 (19.4)	78.3 (21.7)	86.9 (13.5)	81.9 (25.2)		

Maximum Tolerated Dose

The distribution of subjects reaching the maximum dose was similar between treatment groups (Table 3). Twenty-three (82%) adult subjects receiving denufosal and 8 (89%) adult subjects receiving placebo reached the maximum dose for their cohort. Among pediatric subjects, 16 (89%) subjects receiving denufosal and 5 (83%) subjects receiving placebo reached the maximum dose. Of the 8 subjects in the study who did not reach the maximum dose, 7 experienced acute declines in FEV₁ ≥20%, and one experienced clinical symptoms of chest tightness, dyspnea, and wheeze on forced expiration. Six of these 8 subjects had an FEV₁ percent predicted <75% at the screening visit. In pediatric subjects, there was a statis-

tically significant greater decrease in FEV₁ 15 min after the 60-mg dose of denufosal on the first day this dose was given, as compared to placebo ($P = 0.016$). At 2 hr after completion of drug administration, the FEV₁ change was not different from placebo (Fig. 1).

Safety

The percentage of subjects experiencing adverse events was also similar between the denufosal and placebo groups, with most adverse events occurring in the respiratory system (Table 4). Among the adult cohorts, 93% of subjects receiving denufosal and 100% of subjects receiving placebo experienced at least one adverse event, while in the pediatric cohorts, 78% of subjects receiving

TABLE 3—Maximum Tolerated Dose Distribution

	10 mg or placebo, N (%)	20 mg or placebo, N (%)	40 mg or placebo, N (%)	60 mg or placebo, N (%)
Adult subjects				
Cohort 1A				
Denufosal	—	1 (11)	8 (89)	N/A
Placebo	—	—	3 (100)	N/A
Cohort 2A				
Denufosal	—	—	—	10 (100)
Placebo	—	—	—	3 (100)
Cohort 3A ¹				
Denufosal	1 (11)	1 (11)	1 (11)	5 (56)
Placebo	1 (33)	—	—	2 (67)
Pediatric subjects				
Cohort 1B				
Denufosal	1 (11)	—	8 (89)	N/A
Placebo	—	—	3 (100)	N/A
Cohort 2B				
Denufosal	—	1 (11)	—	8 (89)
Placebo	—	1 (33)	—	2 (67)

¹MTD for one active patient could not be determined due to patient being withdrawn prior to determining MTD. NA, not applicable.

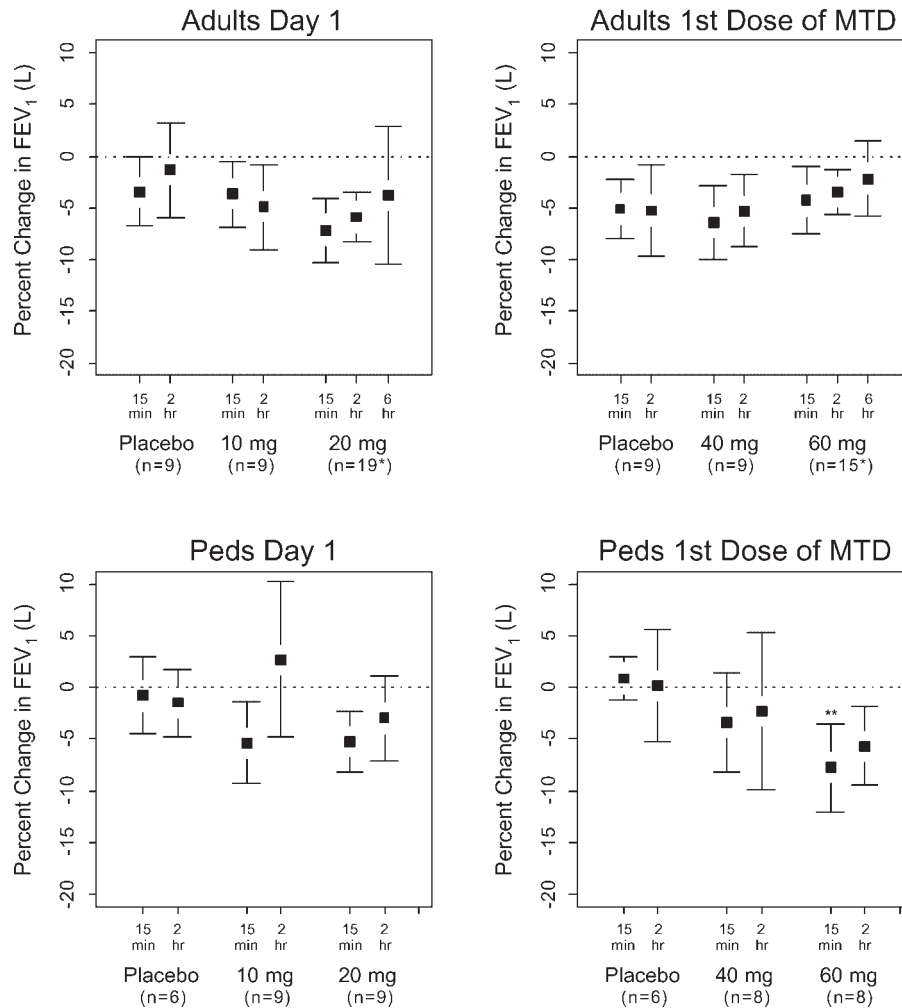


Fig. 1. Mean percent change (and 95% confidence intervals) in FEV₁ from predose to postdose. Plots of FEV₁ response to lower and higher doses of denufosal in adult (Adults) and pediatric (Peds) subjects as compared to placebo. First dose of MTD corresponds to day each subject first received his/her MTD, which in most cases was day 3. Doses of 40 mg and 60 mg were not given on day 1. *N = 9 for 6-hr change on day 1, and N = 5 for 6-hr change on day each subject first received his/her MTD. **P < 0.05, compared to placebo.

TABLE 4—Incidence of Most Frequently Reported Adverse Events by Treatment Group (Number and Percent of Subjects)¹

	Denufosal	Placebo
Adult subjects	N = 28	N = 9
Any adverse event	26 (93%)	9 (100%)
Chest tightness	11 (39%)	3 (33%)
Cough	10 (36%)	3 (33%)
Wheezing	8 (29%)	0
Sputum increased	6 (21%)	5 (56%)
Pediatric subjects	N = 18	N = 6
Any adverse event	14 (78%)	5 (83%)
Cough	10 (56%)	3 (50%)
Headache NOS	4 (22%)	0

¹Most frequently reported adverse events, Defined as those events occurring in more than 20% of denufosal subjects. NOS, not otherwise specified.

denufosal and 83% of subjects receiving placebo experienced an adverse event. The majority (98%) of adverse events in adults were reported to be mild or moderate in intensity, and 71% were reported as being at least possibly related to treatment. Two adult subjects receiving denufosal experienced a serious adverse event of lower respiratory tract infection, with one reported as possibly related to treatment. All pediatric adverse events were mild or moderate in intensity, and 63% were reported as being at least possibly related to treatment. Two adverse events that required hospitalization occurred in this age group, bronchopneumonia (placebo subject) and a new acquisition of *Pseudomonas aeruginosa* infection (denufosal subject), but neither was considered related to treatment.

The most common adverse event in adult subjects was chest tightness, and cough was most common in pediatric

subjects, with similar distributions among treatment groups (Table 4). In adults, wheezing was reported more often in subjects receiving denufosal (29%) compared to subjects receiving placebo (0%), while subjects receiving placebo reported increased sputum more frequently than subjects receiving denufosal (56% vs. 21%). In the pediatric age group, subjects receiving denufosal reported having more headaches than subjects receiving placebo (22% vs. 0%, respectively). Although most adverse events did not tend to be dose-dependent, the majority of wheezing in adults occurred at 60 mg (31% of subjects as compared to 15% in the 40-mg group, 4% in the 20-mg group, and 20% in the 10-mg group). In addition, the incidence of adverse events was higher among subjects with lower lung function ($FEV_1 < 75\%$ of predicted at screening). Seventy-nine percent of subjects with a screening $FEV_1 \geq 75\%$ of predicted and 100% of subjects with a screening $FEV_1 < 75\%$ of predicted experienced at least one adverse event.

FEV_1 profiles immediately following dosing were similar across placebo and denufosal subjects, with some transient decline noted in both groups (Fig. 1). In most cases (67% of FEV_1 measurements across all visits for placebo subjects, and 63% of FEV_1 measurements across all visits for denufosal subjects), FEV_1 values were within 5% of predose values or were improved at 2 hr postdosing. Among adult subjects, the 60-mg dose group had a smaller decline in FEV_1 from predose to 2 hr postdose on day 4 as compared to placebo (-0.08 ± 0.17 l vs. -0.21 ± 0.15 l, respectively; $P = 0.037$). At all other visits and timepoints, FEV_1 values were not significantly different between the placebo and denufosal dose groups. In the pediatric subjects, there was only one significant difference in FEV_1 between treatment groups. The 60-mg dose group had a significant decrease from predose to 15-min postdose on day 3 as compared to placebo (-0.16 ± 0.09 l vs. 0.03 ± 0.05 l; $P = 0.025$). In general, there tended to be mild transient declines in subjects with lower lung function on entry, but these changes were generally not the cause of discontinuation and were observed in both the denufosal and placebo groups (data not shown).

There were no significant differences among the adult dose groups in oxyhemoglobin saturation at any visit. Among pediatric subjects, there was a statistically significant, but clinically negligible change from baseline in oxyhemoglobin saturation at 15 min postdose on day 4 in the denufosal 60-mg dose group compared with placebo (change from baseline, -1.9 ± 1.13 for denufosal 60 mg vs. 0.7 ± 1.75 for placebo; $P = 0.014$). However, this decline in oxyhemoglobin saturation was transient and was improved in the denufosal 60-mg group by the 2-hr postdose timepoint (change from baseline, 0.1 ± 0.83). With this one exception, oxyhemoglobin saturation values were not significantly different between the placebo and denufosal dose groups at any timepoint or visit. Four

(44%) adult subjects receiving placebo and 11 (39%) adult and 4 (22%) pediatric subjects receiving denufosal used a rescue bronchodilator at least once during the study. Other safety assessments, including clinical laboratory tests, vital signs, physical examination findings, and ECGs also showed that study treatments were well-tolerated in both adult and pediatric subjects. No trends were noted in the analysis of shifts from baseline to end of dosing in clinical laboratory values for chemistry, hematology, or urinalysis.

Pharmacological Activity

It was hypothesized a priori that sputum expectoration would provide the best evidence of pharmacological activity of denufosal. Figure 2 depicts the mean change in sputum weight from pre- to postdose for the adult subjects at each visit. Compared to the placebo group, the adult 20-mg dose group had a significant increase in sputum weight on day 1 (-1.12 ± 2.93 g vs. 2.65 ± 5.01 g; $P = 0.019$). After several days of repetitive dosing with denufosal, continued acute sputum production after dosing was not observed, as compared to placebo. Almost half of the pediatric subjects (42%) did not produce any sputum at any of their three visits, and thus these data are not shown. Based on exploratory analyses, it appears that the majority of increased sputum production associated with denufosal treatment was observed in subjects with lower lung function (data not shown).

As might be expected due to the short duration of the trial, no major differences were observed between dose groups for any of the respiratory symptoms contained in the symptom questionnaire.

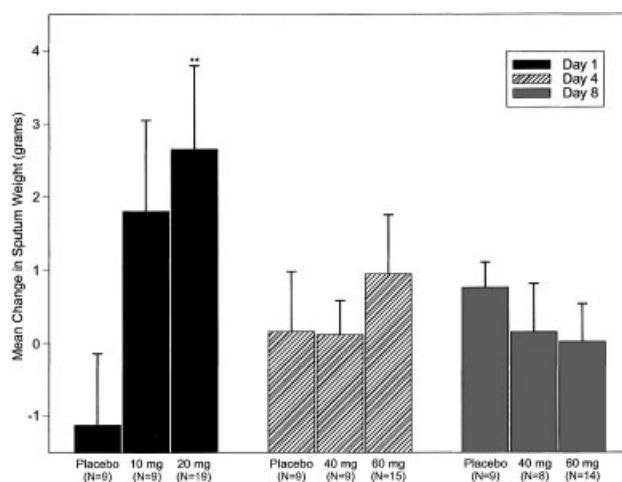


Fig. 2. Mean change in sputum weight from predose to postdose by visit in adult subjects. Error bars represent standard error of mean. Doses of 40 mg and 60 mg were not given on day 1. The 10-mg and 20-mg dose groups are not shown for days 4 and 8, since $N = 1$ and $N = 2$ for these groups, respectively. **** $P < 0.05$, compared to placebo.**

DISCUSSION

Although the survival of patients with CF has improved over the last decade, pulmonary disease continues to be the dominant cause of morbidity and mortality.²⁶ Previous advances in the therapy of CF lung disease focused on “secondary” pathophysiologic events (i.e., infection and inflammation), but recent efforts focused on more proximate (i.e., “primary”) events in the pathophysiologic cascade.²⁷ Specifically, treatment with agents that modify defective ion transport in CF, and the adverse consequences on mucociliary and cough clearance, could conceptually be initiated early in life, and prevent (or slow) the development of lung disease. Aerosolized P2Y₂ agonists would fit this therapeutic paradigm in CF, as these agents stimulate chloride (and liquid) secretion, goblet-cell degranulation, and ciliary beat frequency. Previous studies demonstrated that inhalation of an endogenous P2Y₂ ligand (triphosphate nucleotide, UTP) and an analog (diquafosol, INS365) are safe in normal subjects,^{17,18,20} patients with CF,^{19,21} and patients with airways disease.^{17,22,28} Denufosal (INS37217) is a novel P2Y₂ receptor agonist which may have longer-lasting effects in the airways, since it is metabolically more stable than UTP or diquafosol.⁶

The primary objectives of this current study were to assess the safety and tolerability of single, ascending doses of aerosolized denufosal, and then repeated administrations of the MTD of denufosal, and placebo, in subjects with CF. Secondary objectives were to obtain some preliminary evidence of the pharmacological activity of denufosal, as indexed by sputum expectoration and a symptom questionnaire. This study was designed in conjunction with the Cystic Fibrosis Foundation-sponsored TDN so that a complex study design could be undertaken to address multiple objectives. Specifically, we hoped to study 1) both adult and pediatric subjects with 2) a range of lung dysfunction, so as to identify 3) the MTD by doing single-dose escalation administration, and then 4) assess the safety and pharmacological activity in these different cohorts of study subjects over a 5-day repeated (BID) dosing. Finally, an unusual design feature of the study was the use of a Data Monitoring Committee, whereby studies in adult cohorts were completed, and safety was assessed, before pediatric subjects were studied.

The results of this study indicate that acute administration of denufosal inhalation solution was well-tolerated in both adult and pediatric subjects at doses of up to 60 mg for a single dose, as well as BID dosing for 5 days (Table 4). The frequency of adverse events reported in this study was generally not dose-dependent. For adult subjects receiving denufosal, the most common adverse events were chest tightness, cough, and wheezing. For pediatric subjects, the most common adverse event was cough. Four subjects experienced a serious respiratory adverse event: 3 in the

denufosal group, and 1 in the placebo group. In both pediatric and adult subjects, there was some acute decrease in FEV₁ immediately following dosing, but FEV₁ returned to (or near) predose values after 2–6 hr postdose. It is important to note that this acute (and reversible) change in FEV₁ occurred in a similar pattern in response to inhalation of placebo (normal saline), which implies that (at least) a component of these changes in FEV₁ is not specifically related to drug actions to induce secretion into the airway lumen. Overall, the adverse events suggest that denufosal acts to induce secretion in the airway, associated with a transient reduction in FEV₁, and associated with cough. However, there was no evidence for a sustained reduction in spirometric indices (FEV₁ and FEF_{25%–75%}) in either adult or pediatric subjects after 5 days of repeated (BID) administration. These responses are similar to the acute effects of other P2Y₂ agonists, UTP and diquafosol (INS365), that were tested in normal subjects and patients with CF, primary ciliary dyskinesia, and chronic bronchitis.^{19,22,23,28}

Although this study was designed primarily to assess safety and tolerability, measures of pharmacological activity of denufosal following a single dose and 5 days of repeated dosing were included. The results of this study provide preliminary evidence of the pharmacological activity of denufosal, and provide encouragement about the possible benefits of denufosal as a chronic treatment for CF. There was evidence of a significant treatment-related increase in sputum production relative to baseline compared with placebo following the first dose of denufosal (Fig. 2). This likely reflects the known effects of P2Y₂ receptor agonists on the airways to stimulate liquid secretion and goblet-cell degranulation, and to stimulate ciliary beat frequency. However, this increase in expectorated sputum above baseline conditions was not observed with longer-term, twice-daily dosing. While this could imply a diminution in drug effect with repetitive dosing, this is an unlikely explanation, given what was previously observed with this class of compounds. One possible hypothesis for the observed effect is that chronic dosing with P2Y₂ agonists may induce clearance of excess mucus from the airways on the first day of dosing, and thereby limit the availability of secretions that can be acutely expectorated with repeated dosing. Further studies of longer duration are needed to better understand the impact of denufosal on clearance of airway secretions.

In conclusion, denufosal inhalation solution in doses up to 60 mg was well-tolerated over 3 days of single dosing and 5 days of twice-daily dosing in both adult and pediatric subjects with mild to moderate CF lung disease. Although some acute decline in FEV₁ was noted post-dosing, this occurred in response to both denufosal and placebo, and was reversible. The production of sputum increased with acute dosing of denufosal on the first day of treatment, demonstrating that these doses are pharmacologically active. Based on the results of this study, the

Cystic Fibrosis Foundation-sponsored TDN and Inspire Pharmaceuticals, Inc., recently completed a multicenter 28-day, phase 2 safety and efficacy clinical trial of denufosal inhalation solution in CF subjects with mild ($FEV_1 \geq 75\%$ of predicted) lung disease.

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