Drugs of the Future 2008, 33(8): 668-672

CCC: 0377-8282/2008
DOI: 10.1358/dof.2008.033.08.1237753

Denufosol Tetrasodium

$P2Y_2$ Agonist
Treatment of Cystic Fibrosis

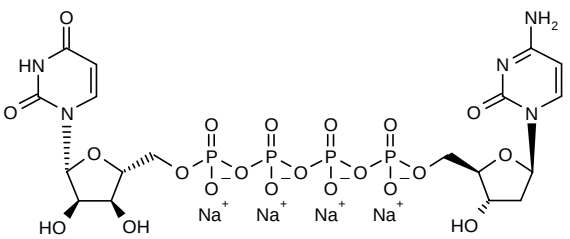
Rec INNM; USAN

INS-37217
dCp4U

P^1-(2'-Deoxycytidin-5'-yl)-P^4-(uridin-5'-yl)tetraphosphate tetrasodium salt

Uridine 5'-(pentahydrogen tetraphosphate) $P'''\rightarrow5'$-ester with 2'-deoxycytidine tetrasodium salt

InChI=1/C18H27N5O21P4.4Na/c19-11-1-3-22(17(28)20-11)13-5-8(24)9(40-13)6-38-45(30,31)42-47(34,35)44-48(36,37)43-46(32,33)39-7-10-14(26)15(27)16(41-10)23-4-2-12(25)21-18(23)29;;;;/h1-4,8-10,13-16,24,26-27H,5-7H2,(H,30,31)(H,32,33)(H,34,35)(H,36,37)(H2,19,20,28)(H,21,25,29);;;;/q;4*+1/p-4/t8-,9+,10+,13+,14+,15+,16+;;;;/m0..../s1



$C_{18}H_{23}N_5Na_4O_{21}P_4$
Mol wt: 861.2504

CAS: 318250-11-2

CAS: 211448-85-0 (free acid)

EN: 291491

Abstract

Cystic fibrosis (CF) is a disease with a low life expectancy and a range of morbidities that result from mutations in the cystic fibrosis transmembrane regulator (*CFTR*) gene. In recent years, investigation has increasingly focused on treating the underlying causes of the disease instead of their downstream effects. One promising approach is to correct abnormal ion transport defects in airways via $P2Y_2$ receptor agonism. One such compound is denufosol tetrasodium (INS-37217), which demonstrated a preclinical pharmacological profile similar to the natural $P2Y_2$ receptor ligand uridine 5'-triphosphate (UTP), increasing chloride and water secretion, ciliary beat frequency and mucin release. *In vivo* enhancement of mucus transport has also been seen, with the agent displaying a notably enhanced duration of action. Denufosol has been safe and well tolerated in clinical studies to date and has demonstrated potentially beneficial effects, including improvements in lung function in CF patients enrolled in a phase II study.

Synthesis

Denufosol can be synthesized as follows:

Uridine 5'-triphosphate (I) is converted to the tributylamine salt, which is then activated as the cyclic metaphosphate (II) employing DCC in DMF. Subsequent coupling of the cyclic phosphate (II) with 2'-deoxycytidine-5'-monophosphate (III) in the presence of tributylamine in DMF gives the title dinucleoside tetraphosphate (1, 2). Scheme 1.

Background

Cystic fibrosis (CF) is a disease for which a comprehensive treatment strategy was not devised until the mid-1960s, and for which survival past childhood has been regarded as a considerable therapeutic achievement. Improvements have been made, but a patient with CF still has a median predicted survival of only 37 years. CF patients suffer malnutrition, frequent respiratory infections, breathing difficulties and permanent lung damage due to the thick mucus which accumulates in their lungs and intestine. Gastrointestinal symptoms result from thick mucus in the pancreas. CF patients are at risk for abnormal heart rhythms and shock resulting from the excessive loss of salt in sweat. CF is also associated with pansinusitis, nasal polyps, allergic bronchopulmonary aspergillosis, digital clubbing, pneumothorax, cor pulmonale, coughing up blood, abdominal pain and discomfort, gastroesophageal reflux and rectal prolapse. Men with CF are typically infertile and women tend to be subfertile. Liver disease, late-onset diabetes, pancreatitis, osteoporosis and gallstones also occur with relative frequency in CF. This morbidity means that CF patients are often hospitalized throughout their lives (3, 4).

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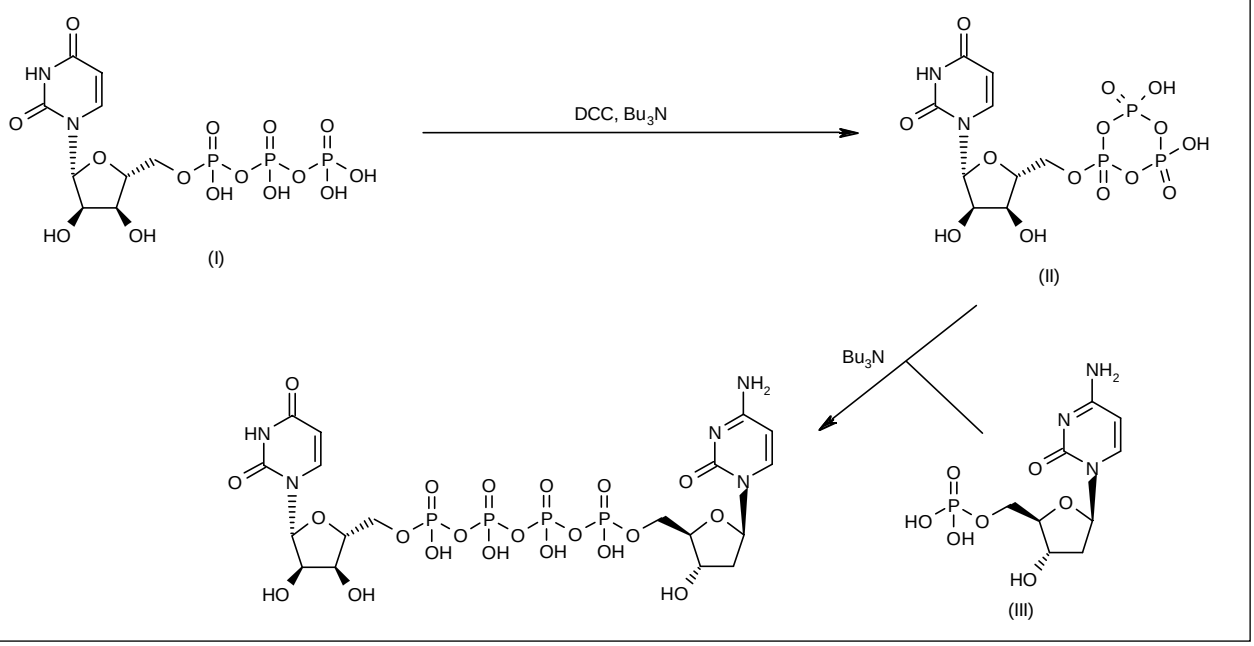
Scheme 1: Synthesis of Denufosol



CF is carried by approximately 3% of the caucasian population as an autosomal recessive trait. The disease affects all races and ethnic groups, but is more common in caucasians. According to the Cystic Fibrosis Foundation, there are approximately 30,000 children and adults with CF in the U.S. and about 70,000 patients worldwide; in the U.S., approximately 1 in 3,500 babies born each year have CF. The disease is caused by mutations in the CF transmembrane regulator (*CFTR*) gene. Defective *CFTR* is associated with abnormal sodium, chloride and water transport across respiratory epithelial cells and the formation of viscous, dehydrated mucus in airways. Impaired mucociliary and cough clearance increases susceptibility to respiratory tract infection (3-7).

The approaches to CF treatment are focused on changing the consistency of mucus, managing pulmonary inflammation or treating bacterial infection. Treatments such as DNAse and inhaled tobramycin have shown benefit in CF patients, but neither affects the underlying cause of CF lung disease. Therapies aimed at targeting the cause of the disease include gene therapy, pharmacotherapy aimed at improving the trafficking, expression or function of *CFTR* and, further along in development, stimulation of chloride secretion via an alternative chloride channel. This last approach, the correction of ion transport defects in the airways, could normalize airways secretions, thereby improving mucociliary clearance and preventing chronic lung infections and progressive lung damage. The activation of $P2Y_2$ receptors on the airways epithelial surface is one means of doing this. The $P2Y_2$ receptor is abundant on the luminal surface of polarized epithelial cells, and $P2Y_2$ agonism has several effects on airways epithelial function, including stimulation of serosal to mucosal chloride and fluid transport, enhancement of mucin secretion from goblet cells, increasing ciliary beat frequency and promotion of surfactant release from type II alveolar cells (8-14).

These effects were seen in research conducted since the early 1990s with uridine 5'-triphosphate (UTP), a natural ligand for $P2Y_2$ receptors. UTP is highly potent but has limited metabolic stability and a relatively short duration of action when administered by inhalation. Efforts were therefore made at Inspire Pharmaceuticals to develop potent and selective $P2Y_2$ agonists with enhanced metabolic stability in CF sputum, yielding INS-365 (diquafosol) and INS-37217 (denufosol tetrasodium). These dinucleotides were also characterized by greater resistance to enzymatic degradation on the airways surface. The superior metabolic stability of denufosol demonstrated in preclinical studies, which is indicative of a longer duration of action, led to its selection for clinical evaluation in CF lung disease, while INS-365 has been developed for dry eye disease, for which it is preregistered (14). Denufosol has also been investigated as a treatment for retinal detachment and macular edema, but this was discontinued in 2006 after two pilot trials failed to demonstrate a reduction in retinal thickness or improvement in visual acuity with the treatment. Denufosol is in phase III development and has orphan drug and fast track status as a treatment for CF in the United States and orphan drug designation in Europe.

Preclinical Pharmacology

A series of experiments were conducted to determine the effects of $P2Y_2$ receptor activation which can increase

mucociliary clearance. Firstly, in rhesus monkey lung, $P2Y_2$ receptor gene expression was detected in bronchial epithelium, including goblet cells, in bronchiolar and alveolar type I and II epithelium and in submucosal gland epithelium. Denufosol was then found to induce mobilization of intracellular calcium in 1321N1 astrocytoma cells stably expressing human $P2Y_2$ and $P2Y_4$ receptors with EC_{50} values of 0.22 and 0.8 μM, respectively. The efficacy of denofusol was the same as that of the endogenous agonist UTP, indicating that it is a full agonist at $P2Y_2$ and $P2Y_4$ receptors, although it was less potent. Denufosol was specific for these receptors, having little or no calcium-mobilizing activity in 1321N1 cells expressing the $P2Y_1$ receptor and only weak activity at $P2Y_6$ receptors (15).

In evaluations of chloride secretion in freshly isolated dog tracheal epithelial preparations, denufosol and UTP stimulated concentration-dependent increases in short-circuit current (I_{sc}) over the concentration range of 0.1-100 μM. Maximal responses to denufosol (at 100 μM) and to UTP (at 10 μM) were similar, and the EC_{50} values for stimulating I_{sc} activity were 1.9 and 0.3 μM, respectively. While both compounds stimulated immediate increases in I_{sc} activity, generally peaking within 7 s of exposure, the response to denufosol was sustained while that to UTP declined soon after peaking (15).

Denufosol concentration-dependently stimulated the production and release of mucin glycoproteins from cultures of human airways epithelium grown in an air/liquid interface (EC_{50} = 2.67 μM). The efficacies of denufosol and UTP were identical in these experiments (15).

In cultured human airways epithelial cells, denufosol stimulated ciliary beat frequency at concentrations above 1 μM, with saturation of effect seen at approximately 100 μM. The peak responses to denufosol were calculated as the ratio of the effect of denufosol to that of the internal control, UTP 100 μM. This yielded an EC_{50} of 8.3 μM for denufosol, and the peak responses to UTP and denufosol at 100 μM were 200% and 180% of baseline, respectively (15).

Denufosol (0.1 mM) proved resistant to airways surface metabolism in human nasal epithelial cells in culture, with a concentration on cell surfaces of approximately 90 μM after 60 min, yielding an average initial hydrolytic rate of 0.02 nmol/min/cm^2. Denufosol was 50 times more stable than UTP and 6 times more stable than the $P2Y_2$ receptor agonist INS-365 on the mucosal surface of these cells. In sputum samples from CF patients, the rate of UTP hydrolysis was comparable to that seen in cultured airways cells. INS-365 and denufosol were more stable in the sputum than on epithelial surfaces, with half-lives of approximately 3 and 25 h, respectively, indicating that sputum would not reduce the effective doses of these agents before reaching their target receptors at the surface of human CF airways epithelial cells (15).

In sheep, denufosol significantly and dose-dependently enhanced tracheal mucus velocity over an 8-h period compared to placebo. The greatest response was seen at 94 μmol, with an increase in tracheal mucus velocity of 160% from baseline seen within 0.25 h. Although similar effects have been observed with earlier $P2Y_2$ agonists, the duration of action of denufosol was notably longer (15).

Pharmacokinetics and Metabolism

Denufosol pharmacokinetics were investigated in a crossover study in 12 healthy volunteers who received 0.1 mg/kg i.v. over 5 min and 200 mg inhaled via the Pari Star nebulizer or 32 mg via nasal spray on separate days. Denufosol plasma concentrations were detectable in all subjects after i.v. administration, with an average maximum concentration of 31.2 ng/ml, which occurred a mean of 5 min after the end of the infusion. In 5 subjects with sufficient plasma samples for the calculation, the half-life was estimated to be < 1 min. After denufosol inhalation, at least one plasma level was detectable in all 12 subjects, and the average maximum concentration was 4.7 ng/ml, with the highest average concentration obtained 2 min after the end of nebulization. Pharmacokinetic analysis was complicated by a low number of samples above the limit of quantification after inhaled administration. Few values exceeded the limits of quantification after intranasal administration and no plasma level exceeded 2.9 ng/ml. It was concluded that systemic exposure of denufosol is low, and the drug is unlikely to have systemic effects after inhaled administration at doses lower than those evaluated here (16).

Safety

The results of toxicology studies of denufosol in rats and dogs indicate that the doses proposed for use in phase III clinical trials are far lower than those that would be unsafe. Local and systemic toxicity was evaluated in Sprague Dawley rats given inhalation administration for at least 26 weeks at pulmonary-deposited dose levels of 0, 3.74, 11.7 and 17.9 mg/kg/day. No treatment-related deaths occurred. Group mean body weight, body weight gain and food consumption declined with mid- and high-dose treatment compared to controls, although these changes were reversible. There were no alterations in ophthalmology, hematology, clinical chemistry, organ weight or macroscopic observations related to denufosol, and the only microscopic change was reversible squamous metaplasia of the ventrolateral larynx epithelium seen with high doses. The no-observed-adverse-effect level (NOAEL) was determined to be 2.69 mg/g lung weight/day, which is at least 6.8 and 14.9 times greater, respectively, than the highest proposed phase III dose in children and adults with CF (17).

Oronasal administration of a nebulized aerosol formulation of denufosol was assessed in beagle dogs at pulmonary-deposited doses of 0, 29.9 and 58.5 mg/kg/day given for 52 weeks. Coughing was seen sporadically but no other toxicity, including changes in lung histopathology, was noted. There were no deaths. The lack of effect on body weight, food consumption, ophthalmology, electrocardiography, respiratory minute volume and other

parameters meant that the NOAEL was set at the high dose of 6.26 mg/g lung weight/day, which is at least 15.7 and 34.8 times greater, respectively, than the highest proposed phase III dose in adults and children with CF (18).

Patients with mild to moderate CF enrolled in a phase I/II study received placebo or single doses of denufosol 10, 20, 40 or 60 mg administered with the Pari LC® Star nebulizer. Repeated dosing was then evaluated with b.i.d. administration of the subject's maximum tolerated denufosol dose or placebo for 5 days. Thirty-seven adults and 24 pediatric patients received at least one dose of study drug. Denufosol was well tolerated in adult and pediatric patients at doses up to 60 mg as a single dose and b.i.d. for 5 days. Among adults, the most common adverse events seen during denufosol administration were chest tightness (39%), cough (36%) and wheezing (29%), and cough (56%) was the most common adverse event in pediatric patients. Three denufosol-treated patients and 1 placebo-treated patient had a serious respiratory adverse event. An acute decline in FEV_1 was seen immediately after dosing in adult and pediatric patients, but FEV_1 levels approached predose levels 2-6 h postdose, and a similar pattern of FEV_1 change was observed in the placebo group (19).

A phase II safety study included CF patients with lower lung function (mean FEV_1 75% of predicted) and patients using concomitant medications, including oral and inhaled antibiotics (N = 72). The randomized, double-blind, multicenter trial evaluated doses of 20 and 60 mg denufosol administered t.i.d. via a standard nebulizer for 28 days. There were two serious adverse events, both pulmonary exacerbations following the treatment phase, one in the placebo group and one in the high-dose denufosol group. The most common adverse event was cough, which occurred in 40% of subjects overall and was comparable across all groups. Patients with lower lung function (FEV_1 = 60-74%) reported more respiratory adverse events across all treatment groups compared to patients with milder disease. Some of these events were acute transient declines in lung function following initial dosing that led to withdrawals, in particular in the 60-mg group (20).

The safety of denufosol in patients with mild CF (median age = 14 years) was assessed in a phase II study in which 89 patients were randomized in double-blind fashion to denufosol inhalation solution in doses of 20, 40 or 60 mg, or placebo. The treatments were administered 3 times daily via the Pari LC® Star nebulizer for 28 days. All but 5 patients completed the study and all but 2 were compliant with treatment. Denufosol was well tolerated, with no dose-related trends in adverse events, the most common of which were cough, headache, pharyngitis and nasal congestion. These adverse events occurred with similar frequency in the denufosol and placebo groups. Cough, the most frequently occurring adverse event, was seen in 52% of placebo-treated patients and 47% of those given denufosol. Adverse events of hemoptysis, decrease in pulmonary function tests, lung infiltration and cough led to the discontinuation of 2 patients in the placebo group, 2 in the denufosol 40 mg group and 1 in the denufosol 60 mg group. An acute, transient decline in FEV_1 2 h after dosing on day 1 was observed in some patients given denufosol, an effect consistent with the drug's mechanism of action. The effect was not significant 5 h postdose. Two serious adverse events (Hodgkin's lymphoma and pulmonary exacerbation) were not considered to be related to treatment. Significantly more waking at night due to cough was seen with denufosol compared with placebo at the end of the study in patients aged 14 or older (29% in the pooled denufosol groups *vs.* 0% for placebo). This is consistent with the activity of denufosol but may have been due to a difference between groups in this parameter at baseline (21).

Clinical Studies

Efficacy data on denufosol are available from some of the clinical studies cited in the Safety section above.

In the phase I/II study in patients with mild to moderate CF discussed above, the primary objective was to assess safety and tolerability, but one measure of pharmacological activity was also determined. In adults given denufosol 20 mg, a significant increase in sputum weight was seen after the first dose compared with placebo (2.65 g *vs.* –1.12 g). Acute sputum production was not seen, however, after several days of repeated dosing, which may have been due to the clearance of excess mucus from the airways on the first day, reducing the amount of secretions which could later be expectorated. A symptom questionnaire revealed no differences between treatment groups for respiratory symptoms (19).

The first solid evidence of a treatment benefit with denufosol in patients with CF was obtained in the phase II study which evaluated inhaled doses of 20, 40 and 60 mg. Effects on measures of air flow obstruction were seen with denufosol, with significant differences in FEV_1, forced expiratory flow at 25-75% of vital capacity ($FEF_{25-75\%}$), FVC and FEV_1/FVC for the pooled denufosol groups compared to placebo. In analyses by dose, a significantly greater change from baseline in FEV_1 was seen with denufosol 20 and 60 mg compared to placebo, with differences from placebo of 0.18 and 0.15 l, respectively. Similarly, significantly greater changes in $FEF_{25-75\%}$ were seen with denufosol 20 and 60 mg, with differences from placebo of 0.40 and 0.30 l/s, respectively. The same was true for FVC, with differences from placebo of 0.12 and 0.13 l, respectively, while a significant effect on FEV_1/FVC was seen only with the 20-mg dose (difference from placebo 0.03). These findings were adjusted for study site, baseline spirometry values, age, sex and *Pseudomonas aeruginosa* status. There were no treatment differences on high-resolution computed tomography lung scan parameters. Overall, the improvement in air flow mechanics was modest, which may have been related to the mild lung impairment in the study population at baseline (21).

Top-line results from a phase III study (Transport of Ions to Generate Epithelial Rehydration, TIGER-1) comparing denufosol and placebo in patients with mild CF revealed a statistically significant improvement in FEV_1

from baseline with denufosol, with a 45-ml treatment group difference compared to placebo. The randomized, double-blind study included 352 patients and consisted of a 24-week, placebo-controlled treatment period followed by an open-label safety assessment. While patients given denufosol generally had improved FEV_1, patients given placebo showed essentially no change. The treatment effect of denufosol increased over the 24-week placebo-controlled period and preliminary data in 210 patients completing the extension indicated that FEV_1 continued to improve during weeks 24-48. A trend in differences in $FEF_{25-75\%}$ also favored denufosol, while no significant differences between denufosol and placebo were seen in pulmonary exacerbations. Denufosol was well tolerated and at the time of reporting, analysis of prespecified secondary endpoints and subgroups was ongoing (22, 23).

TIGER-2, a second phase III study, has been initiated. The randomized, double-blind study is intended to enroll approximately 350 patients who will receive denufosol 60 mg t.i.d. or placebo for 24 weeks (24). A phase II trial in patients with mild to moderate CF is also under way (25).

Source

Inspire Pharmaceuticals, Inc. (US).

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