P2Y<sub>2</sub> Receptor Agonists

A New Class of Medication Targeted at Improved Mucociliary Clearance

Donald J. Kellerman, PharmD

Chronic bronchitis is in part characterized by mucus hypersecretion and the inability to clear airways of mucus. Despite years of research in this area, to date there are no pharmacologic therapies available to enhance or promote mucociliary clearance. P2Y<sub>2</sub> receptor agonists are a new class of mucolytic compounds that are currently under development for this purpose. This article will review the pharmacology of P2Y<sub>2</sub> receptor agonists, review the clinical studies performed to date, and highlight the challenges inherent in the development of therapies with these pharmacologic properties.

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Key words: COPD; chronic bronchitis; cystic fibrosis; INS365; mucociliary clearance; purinergic receptors; P2Y receptors; uridine triphosphate

Abbreviations: ATP = adenosine triphosphate; CF = cystic fibrosis; MCC = mucociliary clearance; UTP = uridine triphosphate

Mucus hypersecretion and the inability to clear mucus from the airways are cardinal manifestations of chronic bronchitis. Not only is mucus hypersecretion associated with breathlessness and cough, but this generally copious and tenacious mucus is thought to promote infection and lung damage. If a therapeutic agent could be developed that helped patients with chronic bronchitis “clear their lungs,” either by enhancing the breakdown of mucoproteins and/or by reducing the viscosity of the mucus, the intensity of the symptoms and the frequency of the pulmonary exacerbations could be reduced.

This article is a review of a new class of compounds, P2Y<sub>2</sub> receptor agonists, that are currently under development for the treatment of a variety of conditions in which mucociliary clearance (MCC) is impaired, including chronic bronchitis and cystic fibrosis (CF). Although it is early in the clinical development of these compounds, P2Y<sub>2</sub> agonists have shown promise in that they improve MCC in smokers with impaired clearance. The studies that have been performed to date will be reviewed, as well as challenges in developing a compound with these pharmacologic properties.

In the past, a number of pharmacologic approaches that have been aimed at addressing the problem of mucus hypersecretion in patients with chronic bronchitis have been evaluated. Early guidelines for the treatment of patients with chronic bronchitis recommended the use of expectorants such as a saturated solution of potassium iodide as part of the therapeutic regimen. A variety of trials have evaluated the effectiveness of N-acetylcysteine (Mucomyst; Apothecon; Plainsboro, NJ), both via inhalation and orally, for the treatment of patients with chronic bronchitis. None of these trials have shown a clear-cut benefit for N-acetylcysteine or other similar compounds in the treatment of patients with chronic bronchitis.1,2

One of the most ambitious trials evaluating the efficacy of a mucolytic compound was the National Mucolytic Study.11 In this 8-week study of 361 patients with chronic bronchitis, the efficacy of iodinated glycerol tablets was compared to placebo. The primary efficacy assessments were based on a questionnaire of common chronic bronchitis congestion symptoms. For some of the measures evaluated in the trial, the results were favorable for iodinated glycerol. However, the formulation tested in this trial was later withdrawn from the market and is no longer commercially available.

Expectorants such as guaifenesin are sometimes prescribed for patients in hopes of providing some therapeutic benefit; however, the evidence for the efficacy of these treatments is lacking. Currently, there are no mucolytic compounds recommended by treatment guidelines for patients with chronic bronchitis.1,2,3

Thus, despite decades of work and the performance of multiple, large, well-designed clinical trials, there are no pharmacologic agents available to address one of the fundamental disorders in chronic bronchitis, that of mucociliary impairment. This constitutes a large unmet medical need and underscores the enthusiasm for the development of P2Y<sub>2</sub> receptor agonists for the treatment of chronic bronchitis and other respiratory conditions in which MCC is impaired.

Pharmacology of P2Y<sub>2</sub> Agonists

The role of purinergic receptors and the description of their pharmacology were originally proposed by Burnstock and colleagues4,14,15 by way of describing responses that previously had been defined as nonadrenergic and noncholinergic. Adenosine triphosphate (ATP) was proposed as the natural ligand of these receptors. On further study, it was proposed that purinergic receptors should be subdivided into P1 receptors, which respond to adenosine and are coupled to adenylyl cyclase, and P2 receptors, which respond to ATP and adenosine diphosphate (Fig 1). The P2 class subsequently has been divided further into ion channels (P2X) and G protein-coupled P2Y receptors. These receptors, which are found on the apical surface of airway epithelia, are believed to be the major coordinator of the MCC mechanism in the lung16 and, thus, have been the most extensively studied.

The natural ligand of P2Y<sub>2</sub> receptors is uridine triphosphate (UTP). UTP stimulates serosal-to-mucosal chloride transport and fluid transport via a non-CF transmembrane regulator mechanism in isolated normal and CF epithelial cells.17–19 Subsequently, it was found that UTP stimulated mucin secretion from goblet cells,20 increased surfactant

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Figure 1. Purinergic receptor superfamily tree. The pharmacologic lineage of the P2Y2 receptor subtype is derived from the P2 receptors, which respond to nucleotides. Ado = adenosine; GPCR = G-protein coupled receptor.

Table 1—Major Actions of P2Y2 Agonists

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<th>Action</th>
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<tr>
<td>Increase chloride and water secretion</td>
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<td>Increase mucus release from goblet cells</td>
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<tr>
<td>Increase cilia beat frequency</td>
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<tr>
<td>Stimulate release of surfactant from type II alveolar cells</td>
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release from type II alveolar cells, increased the beat frequency of cilia in isolated normal and CF epithelial cells. Thus, the activation of the P2Y2 receptors can stimulate the major components of the MCC system (Table 1).

Although it was assumed that P2Y2 receptors resided in the respiratory epithelia, only recently was it confirmed using in situ hybridization that there is a message for P2Y2 receptors in the respiratory epithelia of primates. This provided additional evidence that the P2Y2 system plays a role in activating the MCC system as part of the body's natural defense mechanism. Studies in various in vitro systems have shown the ability of the P2Y2 agonists to enhance the components of the MCC system.

Further studies in whole animals were deemed to be appropriate. A commonly used model of MCC involves the measurement of tracheal mucus velocity in sheep. This model permits one to measure the rate of mucus transport in a single large airway, and this correlates well with whole-lung MCC. Using the sheep model, P2Y2 agonists had a significantly greater effect than saline solution in enhancing tracheal mucus velocity. Confirmation studies had a significantly greater effect than saline solution in increasing the amount of sputum produced by smokers, and the effect appeared to be dose-related. To evaluate whether UTP could enhance MCC, technetium-labeled iron oxide particles were inhaled, and their clearance was measured using gamma scintigraphy. Nebulized UTP doses of 20 and 100 mg significantly increased the MCC rate compared to that at baseline or after the administration of saline solution (Fig 2).

Another study demonstrated that UTP could increase lung clearance in patients with primary ciliary dyskinesia. This was a particularly interesting finding, since ciliary function would not contribute to the increased clearance in these patients, suggesting that cough clearance can be enhanced by the use of P2Y2 agonists.

Although these findings were encouraging, there was also evidence that was accumulated at the time about the shortcomings of UTP as a potential therapeutic agent. Studies performed examining the metabolism of UTP in cultures of human bronchial epithelial cells or CF sputum demonstrated that UTP was broken down to non-P2Y2 agonists in a matter of minutes. This led to a search for more metabolically stable compounds, which ultimately led to the synthesis of compound INS365 (Inspire Pharmaceuticals, Durham, NC) [Fig 4]. INS365 is now being evaluated as a therapeutic agent for the treatment of dry eye disease (ie, keratoconjunctivitis sicca) and chronic bronchitis.

INS365 was evaluated in preclinical models that were similar to those used previously for UTP, including the sheep model of MCC. The compound showed P2Y2 agonist activity, as well as enhanced metabolic stability in vitro with a prolonged half-life in cultures of both human bronchial epithelial cells and sputum from CF patients. The initial evaluation of INS365 in humans was performed as a rising-dose evaluation in 75 healthy nonsmokers and cigarette smokers. Single nebulized doses were administered, and volunteers were closely monitored. INS365 doses up to 400 mg were generally well-tolerated in nonsmokers, whereas cigarette smokers were able to tolerate doses only up to 100 mg. The most common adverse event, mild-to-moderate coughing, often resulted in sputum production and was consistent with the mechanism of action. The effect on increasing sputum production was dose-related in smokers. There were no serious adverse events.
As with UTP, the effects of inhaled INS365 on MCC in smokers was evaluated using inhaled radiolabeled iron oxide particles as the marker. This study compared 40 and 80 mg INS365 with placebo and the baseline period in 12 cigarette smokers. In this study, both doses of INS365 significantly increased the rate of MCC, compared to that after administration of inhaled saline solution or the baseline period (Fig 5). Thus, like UTP, INS365 administration increased MCC in smokers.

The tolerability of the INS365 inhalation solution also has been evaluated in adults and children with CF. An ascending single-dose tolerability study in 84 subjects was recently completed. In this study, doses of 20, 40, 80, and 100 mg were evaluated in adults, and doses of 20, 40, and 80 mg were evaluated in children. At baseline, subjects had an FEV₁ > 45% and were clinically stable with no evidence of acute respiratory tract infections or current pulmonary exacerbations.

Due to the expected pharmacologic action of INS365 and the experiences in smokers, the criteria for intolerability were prospectively defined based on decreases in lung function and falls in oxyhemoglobin saturation. Doses of INS365, 20 and 40 mg, were well-tolerated in both adults and children. As was observed in healthy smokers, there was a dose-related increase in expectorated sputum in adults (Fig 6) and a dose-related increase in cough. The incidence of cough and increased sputum production was sometimes accompanied by a modest and transient decline in FEV₁. The average sputum production for the six adult patients with significant falls in their FEV₁ was 8.9 g compared to saline solution.
to 3.9 g for patients without FEV1 changes. These observations are consistent again with the expected pharmacologic action of the drug. Although pediatric patients experienced a dose-related incidence of cough, they produced little sputum before or after treatment, as is typical with children with CF.

**Challenges in Developing P2Y2 Agonists**

As was alluded to in the introduction, the clinical development of mucoactive drugs for chronic bronchitis has been marked by several ambitious efforts without success. Perhaps the paramount challenge is that there are currently no direct, noninvasive objective methods with which to assess the quantity, character, and distribution of airway secretions in humans. As a result, indirect measures such as sputum volume, lung function, and MCC have been used as surrogate markers. These indirect measures, however, have limitations. For example, an increase in sputum expectoration in response to a therapy does not necessarily mean that there has been a commensurate decrease in the amount of secretions in the airways. Additionally, lung function can be affected by multiple factors, of which mucus hypersecretion is only one.

A number of factors can influence patients’ responses to treatment. The effects of mucoactive compounds, although believed to be desirable and ultimately beneficial, may be associated with transient paroxysms of cough and transient breathlessness, particularly at higher doses, as a direct result of their pharmacologic effects. It remains a challenge to select appropriate doses that achieve the desired mucoactive effects while maximizing patient tolerability.

As was shown in some of the early studies, sputum production can be significantly increased if the dose of a P2Y2 agonist is increased. However, acute dramatic increases in sputum production may not be desirable in patients with preexisting mucus gland hypertrophy and the potential for mucus plugging. Thus, considerable attention will need to be given to determining a dose response that has a beneficial effect on MCC without significant untoward effects on pulmonary function.

Finally, consideration must be given to the selection of appropriate subjective end points for mucoactive drugs. As this class of compounds can have negative effects on pulmonary function, particularly in the short term, pulmonary function measured immediately after dosing does not seem to be an appropriate efficacy end point. Alternatively, the primary assessment of efficacy probably should be a diary instrument that has been developed and validated to assess meaningful changes in symptoms and/or quality of life that are important to patients. In long-term studies, the reduction in frequency or severity of clinical exacerbations may prove to be another quantifiable and undoubtedly clinically meaningful end point.
CONCLUSION

In conclusion, P2Y2 receptor agonists are a new class of compounds that are being developed for the treatment of a variety of conditions in which MCC is impaired, including chronic bronchitis and CF. Early research to date shows promise that dose-related increases in MCC and sputum production can be achieved with either UTP or INS365. Although the benefits of enhanced MCC will likely be improved symptoms and possibly even lower long-term exacerbation rates, the short-term pharmacologic actions of the drug induce cough and sputum production. As these effects can be associated in some individuals with transient breathlessness, a careful balance must be achieved between patient tolerability and the beneficial effects of enhanced MCC.

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