Efficacy Testing of Montelukast Loaded Large Porous Particles in Allergen-Induced Rat Asthma Model

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ABSTRACT SUMMARY
In this study, we investigated the efficacy of respirable large porous PLGA particles of montelukast in alleviating the symptoms of asthma in a rat model. PLGA particles of montelukast were prepared using a double emulsion-solvent evaporation method and the porosity was adjusted by incorporating polyethyleneimine (PEI), as a porosigen. Large porous particles (PEI-1) showed acceptable physicochemical properties and favorable release profiles for pulmonary administration. Compared to non-porous particles, PEI-1 showed reduced uptake by alveolar macrophages and increased deposition onto rat lungs. PEI-1 significantly reduced infiltration of inflammatory cells in the airways and narrowing of airway lumens in asthmatic rats. Overall, the preliminary data suggest that inhaled prolonged release montelukast particles could be used as an alternative to oral form of the drug.

INTRODUCTION
Montelukast, an orally administered leukotriene receptor antagonists (LTRA), is commonly used as a second-line treatment for the management of inhaled corticosteroids resistant asthma in chronic asthmatic patients. It acts as a potent antagonist selectively at type 1 cysteinyl leukotriene receptors (CysLT1,R). Montelukast shows its primary anti-inflammatory effects that prevent CysLTs-induced recruitment and activation of inflammatory cells and reduce their pro-inflammatory priming activities through interaction with CysLT1,R. Interestingly, recent findings suggest that at higher concentrations, montelukast shows a series of secondary anti-inflammatory activities [1], which are seemingly distinct from those normally observed upon blockage of CysLT1,R. We hypothesize that montelukast loaded particles administered via the inhalation route directly into the lungs produce therapeutic drug concentrations for a long time locally and ameliorates the inflammation associated lung disorders.

EXPERIMENTAL METHODS

Preparation and physicochemical characterization of particles: Six lots of montelukast loaded particles were prepared by varying polymer type (PLA, PLGA 85:15 and PLGA 50:50) and adding porosigen (none or PEI). Particles were characterized according to our published procedure with slight modification [2]. Entrapment efficiency was measured by quantifying the drug extracted from particles in methanol using an HPLC method. In vitro drug release of the drug was studied in simulated lung fluid (SLF) as per published procedure [2, 3]. An optimized porous particulate formulation (PEI-1) and a non-porous formulation (N-2), used as a control, were used subsequently in various cellular and in vivo studies.

Particle uptake by rat alveolar macrophages was performed by incubating fluorescent particles with alveolar macrophages collected from rat lungs as described previously [2].

Particle deposition in rat lungs was studied by administering porous and non-porous fluorescent particles via the pulmonary route followed by imaging using IVIS whole body animal imager [2].

Safety studies were performed by measuring total proteins and injury markers (ALP and LDH) in bronchoalveolar lavage fluid (BAL) collected 12 h after the treatment [2].

Efficacy studies were performed in ovalbumin-induced rat asthma model [4]. Asthmatic rats were divided into 3 groups to receive no treatment, montelukast or PEI-1. Healthy rats were used as negative control. Lungs were harvested and BAL fluid was collected 24 h post administration. Following fixing, lungs were sliced and sections were stained with hematoxylin and eosin to assess narrowing of airway lumen. BAL fluid was analyzed for total protein content and inflammatory cells infiltration.

<table>
<thead>
<tr>
<th>Microparticulate formulations</th>
<th>N-2 (Non-porous)</th>
<th>PEI-1 (Porous)</th>
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<tbody>
<tr>
<td>Composition</td>
<td></td>
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<tr>
<td>Porosigen</td>
<td>None</td>
<td>PEI</td>
</tr>
<tr>
<td>Polymer</td>
<td>PLGA (85:15)</td>
<td>PLGA</td>
</tr>
<tr>
<td>Particle size</td>
<td>2.27 ± 0.02</td>
<td>7.72 ± 0.07</td>
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<tr>
<td>Tapped Density</td>
<td>0.43 ± 0.02</td>
<td>0.10 ± 0.02</td>
</tr>
<tr>
<td>MMAD</td>
<td>1.49 ± 0.04</td>
<td>2.47 ± 0.03</td>
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<tr>
<td>Zeta potential</td>
<td>-8.13 ± 0.06</td>
<td>7.61 ± 1.97</td>
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<tr>
<td>% Drug entrapment</td>
<td>89.32 ± 1.29</td>
<td>82.52 ± 0.41</td>
</tr>
</tbody>
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Table 1: Composition and physicochemical properties of montelukast microparticles

RESULTS AND DISCUSSION
It is feasible to prepare large porous PLGA particles of montelukast, a hydrophobic drug. Similar to previous report, addition of 3% PEI in the internal phase resulted in porous particles [5]. Physicochemical characterization revealed that PEI-1 particles were large in size with significantly reduced density and positive surface charge (Table 1). Montelukast was efficiently encapsulated into the particles exhibiting >80%...
entrapment efficiency. Further, ~35% and 30% drug was released in a controlled fashion in SLF over 7 days from PEI-1 and N-2, respectively. In vitro drug release profiles showed minimal initial burst release from the particles (Fig. 1A).

Owing to their relatively large size, fluorescent PEI-1 particles showed decreased uptake when incubated with rat alveolar macrophages compared to smaller non-porous N-2 particles (Fig. 1B). Further, due to large size and low density, PEI-1 porous particles demonstrated substantially increased deposition in rat lungs compared to smaller and denser non-porous N-2 particles (Fig. 1C). Also, PEI-1 particles were safe considering the fact that no increase in the levels of injury markers was observed in BAL fluid after pulmonary administration.

Administration of PEI-1 to asthmatic rats significantly reduced activation and infiltration of inflammatory cells, especially eosinophils, from the blood stream into the airways (Fig. 2A) due to sustained release of drug from the particles. Further, compared to asthmatic rats that received plain montelukast, large porous particles significantly improves the histopathological changes such as thickening of airway walls (Fig. 2B) and goblet cell hyperplasia that occurs in asthmatic condition.

CONCLUSION
Montelukast encapsulated in large porous particles demonstrated heightened anti-asthmatic efficacy compared to plain drug given via the pulmonary route. The proposed formulations are likely to alleviate the symptoms of asthma and reduce the systemic exposure of the drug that is associated with oral montelukast.

REFERENCES: