# Feasibility Study of Aerosolized Prostaglandin $E_1$ Microspheres as a Noninvasive Therapy for Pulmonary Arterial Hypertension

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**ABSTRACT:** This study was designed to test the feasibility of polymeric microspheres as an inhalable carrier for prostaglandin $E_1$ ($PGE_1$) for treatment of pulmonary arterial hypertension. Poly(lactic-*co*-glycolic acid) (PLGA) microspheres were prepared by a double emulsion–solvent evaporation method. Six different microspheric formulations were prepared using two different blends of PLGA (50:50 and 85:15) and varying concentrations of polyvinyl alcohol (PVA) in the external aqueous phase (EAP). The particles were characterized for morphology, size, aerodynamic diameter, entrapment efficiency, release patterns, and metabolic stability. Pulmonary absorption was studied in a rat model, and safety of the formulations was evaluated by measuring cytotoxicity in Calu-3 cells and assessing injury markers in bronchoalveolar lavage (BAL) fluid. Both actual particle size and aerodynamic diameter of the formulations decreased with increasing PVA concentration. The mass median aerodynamic diameter of the particles was within the respirable range. Entrapment efficiency increased with increasing PVA concentration; PLGA 85:15 showed better entrapment due to hydrophobic interactions with the drug. Compared to intravenously administered $PGE_1$, microspheres prepared with PLGA 85:15 produced a 160-fold increase in the half-life of $PGE_1$ following pulmonary administration. Although plain $PGE_1$ showed rapid degradation in rat lung homogenate, $PGE_1$ entrapped in the particles remained intact for about 8 h. Optimized formulations were demonstrated to be safe, based on analysis of cytotoxicity and lung-injury markers in BAL fluid. Overall, the data suggest that microspheric $PGE_1$ formulations have the potential to be used as a noninvasive and controlled-release alternative to the current medications used for treatment of pulmonary arterial hypertension that are administered by continuous infusion or require multiple inhalations.  J Pharm Sci 99:1774–1789, 2010

**Keywords:** pulmonary arterial hypertension (PAH); prostaglandin $E_1$ ($PGE_1$); PLGA microspheres; polyvinyl alcohol (PVA); pulmonary delivery

## INTRODUCTION

Pulmonary arterial hypertension (PAH) is a chronic and debilitating disorder of the pulmonary circulation that affects about 50,000–100,000 people in the United States.[1] PAH is characterized by mean pulmonary arterial pressure (MPAP) above 25 mmHg at rest or 30 mmHg after exercise. The primary cause of

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PAH progression is an imbalance of neurochemical mediators—prostacyclins, nitric oxide, endothelin-1—that are required to maintain the vascular tone of pulmonary arteries. This imbalance causes vasoconstriction, pulmonary vascular remodeling, and increased pulmonary vascular resistance that result in occlusion and narrowing of the pulmonary arteries and eventually right ventricular enlargement.[2] Development of PAH is associated with a reduction in prostacyclin levels in the pulmonary circulation.[3] Various prostacyclin $I_2$ ($PGI_2$) analogs, including epoprostenol, iloprost, and treprostinil, are currently available for PAH treatment. However, because of their very short biological half-lives, prostacyclin analogs are required to be administered by continuous intravenous or subcutaneous infusion. Use of indwelling catheters to administer the drugs results in a variety of side effects, including infection and cardiovascular collapse.[4] To overcome these limitations, an inhalable prostacyclin analog (iloprost, Ventavis®) has been developed and is now commercially available in the United States.[5] An inhalable form of treprostinil has recently been tested in humans.[6,7] However, the limitations of very short biological half-life and metabolic instability continue to be major limitations for PAH therapy. Inhaled iloprost is required to be taken 9–12 times a day, which often leads to patient noncompliance.

Prostaglandin $E_1$ (alprostadil, $PGE_1$) is one of the prostacyclins that has been investigated for PAH therapy.[8,9] $PGE_1$ acts as a selective pulmonary vasodilator when administered as an aerosol into the lungs and thereby eliminates the complications associated with systemic vasodilation.[10] In fact, intravenous $PGE_1$ has been reported to be used for treatment of PAH, acute respiratory distress syndrome, hypoxemic respiratory failure, and in lung transplantation.[11,12] However, it has a half-life of 5–10 min because 70–90% of the drug metabolizes in the lungs in a single pass. Moreover, because of its extensive peripheral distribution, intravenous $PGE_1$ is associated with severe side effects such as systemic hypotension and low cardiac output.[12] The limitations of $PGE_1$-based PAH therapy can be overcome by delivering the drug as long-acting inhalable particles. Approaches that have been used to increase the residence time of $PGE_1$ in the lungs include: (i) chemical modification,[13] (ii) formulation in a β-cyclodextrin complex,[14] and (iii) encapsulation in particulate carriers such as lipids, microspheres,[9,15] liposomes,[16] and other polymeric carriers.[17] Further, $PGE_1$ is reported to be continually released upon encapsulation in PLGA particles.[18,19] In fact, PLGA-based particulate carriers have been extensively studied for oral and nasal delivery of various large and small molecular weight drugs.

Recently, we showed that PLGA microspheres can be used to achieve prolonged release of low molecular weight heparins after pulmonary administration.[20] Incorporation of $PGE_1$ into microspheric carriers is likely to offer the following advantages: (i) prevent inactivation by phase-I metabolic enzymes in the lungs, (ii) release the drug over a longer period of time and produce a prolonged therapeutic effect, and (iii) minimize the distribution of $PGE_1$ in the body by localized delivery of the drug in the lungs. However, the pulmonary route suffers from some important limitations such as poor deposition of particles that are outside the respirable size range (1–5 μm) and loss of drug due to oropharyngeal deposition. The problem of poor deposition in the respiratory tract can be addressed by encapsulating the drug in particles with mass densities $<0.4\,g/cm^3$ and geometric diameter $>5\,\mu m$, as proposed by Edwards et al.[21] This approach will facilitate respirability, enhance deep-lung deposition, and produce prolonged release by avoiding uptake by alveolar macrophages. This study therefore tests the hypothesis that $PGE_1$-loaded PLGA-based microspheres, when administered via the pulmonary route, release the drug over a prolonged period of time, and prevent enzymatic degradation of $PGE_1$ in the lungs.

## MATERIALS AND METHODS

### Materials

PLGA 50:50 with inherent viscosity 0.55–0.75 dL/g (average molecular weight = 43.5 kDa) and PLGA 85:15 with inherent viscosity 0.55–0.75 dL/g (average molecular weight = 85.2 kDa) were purchased from Boehringer Ingelheim (Lactel Absorbable Polymers, Pelham, AL). $PGE_1$ was purchased from Cayman Chemical Company (Ann Arbor, MI). Polyvinyl alcohol (PVA) and dichloromethane (DCM) were from Sigma–Aldrich, Inc. (St. Louis, MO) and VWR International (West Chester, PA), respectively.

### Preparation of $PGE_1$-Loaded PLGA Microparticles

$PGE_1$-loaded PLGA microparticles were prepared by the water-in-oil-in-water (W/O/W) double