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Inhaled sildenafil as an alternative to oral sildenafil in the treatment of pulmonary arterial hypertension (PAH)

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ABSTRACT

The practice of treating PAH patients with oral or intravenous sildenafil suffers from the limitations of short dosing intervals, peripheral vasodilation, unwanted side effects, and restricted use in pediatric patients. In this study, we sought to test the hypothesis that inhalable poly(lactic-*co*-glycolic acid) (PLGA) particles of sildenafil prolong the release of the drug, produce pulmonary specific vasodilation, reduce the systemic exposure of the drug, and may be used as an alternative to oral sildenafil in the treatment of PAH. Thus, we prepared porous PLGA particles of sildenafil using a water-in-oil-in-water double emulsion solvent evaporation method with polyethyleneimine (PEI) as a porosigen and characterized the formulations for surface morphology, respirability, in-vitro drug release, and evaluated for in vivo absorption, alveolar macrophage uptake, and safety. PEI increased the particle porosity, drug entrapment, and produced drug release for 36 h. Fluorescent particles showed reduced uptake by alveolar macrophages. The polymeric particles were safe to rat pulmonary arterial smooth muscle cell and to the lungs, as evidenced by the cytotoxicity assay and analyses of the injury markers in the bronchoalveolar lavage fluid, respectively. Intratracheally administered sildenafil particles elicited more pulmonary specific and sustained vasodilation in SUGEN-5416/hypoxia-induced PAH rats than oral, intravenous, or intratracheal plain sildenafil did, when administered at the same dose. Overall, true to the hypothesis, this study shows that inhaled PLGA particles of sildenafil can be administered, as a substitute for oral form of sildenafil, at a reduced dose and longer dosing interval.

1. Introduction

Sildenafil, a drug used in the treatment of erectile dysfunctions, works via nitric oxide mediated relaxation of penile smooth muscles [1–4]. Sildenafil competitively inhibits enzyme phosphodiesterase type 5 (PDE5), which inactivates cyclic guanosine monophosphate (cGMP). Just as in the penile smooth muscles, PDE5 is also highly expressed in pulmonary arterial smooth muscle cells (PASMCs) [5,6]. Because of high expression of PDE5 in human PASMCs, sildenafil reduces pulmonary arterial pressure in patients with pulmonary arterial hypertension (PAH). Sildenafil, now approved for its use in adult PAH patients [7–9], reduces pulmonary arterial pressure by increasing the levels of cGMP and nitric oxide in the pulmonary vasculature [10]. Since its approval for use in PAH in 2005, sildenafil has become a widely prescribed anti-PAH drug and an important member of the three major categories of anti-PAH medications that include prostanoids, endothelin receptor antagonists, and PDE5 inhibitors [8,9].

Currently, sildenafil is administered orally (tablets) or intravenously for the treatment of PAH [10,11]. However, the use of oral or intravenous sildenafil in PAH is associated with some practical limitations including a large dose, short dosing-intervals, unwanted systemic side-effects due to systemic exposure and limited use in pediatric populations [12–14]. Indeed, long-term use of oral/intravenous sildenafil causes resting hypotension and nose-bleeding, elicits painful and prolonged penile erections, and worsens pulmonary vascular occlusive disorders [15]. Moreover, chronic use of sildenafil is not recommended in PAH afflicted children [16,17].

We believe that many of the limitations of oral sildenafil can be overcome by reducing the dose and dosing frequency of the drug. In fact, as an alternative to the oral form of the drug, poly-lactic-*co*-glycolic acid (PLGA) particles of sildenafil have been prepared with a goal to treat PAH and other diseases [18,19]. Nebulized sildenafil has been reported to potentiate the vasodilatory effects of nitric oxide in a sheep model

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of PAH [20]. However, no studies have systemically evaluated the feasibility of aerosolized formulations of sildenafil, nor have any published studies shown the advantages of inhaled prolonged-release sildenafil over oral sildenafil. In this study, we proposed to test the hypothesis that inhaled long-acting particulate formulations of sildenafil produce pulmonary preferential vasodilation at a reduced dose and dosing frequency, and reduce systemic drug exposure.

With this goal in mind, we prepared porous PLGA polymer based inhalable microparticles of sildenafil citrate by a water-in-oil-in-water (w/o/w) double emulsion solvent evaporation method. Polyethyleneimine (PEI) was used in the internal aqueous phase (IAP) as a porosigen. Sildenafil loaded particles were characterized for surface morphology, particle size, zeta potential, drug loading efficiency, aerodynamic properties, drug release in a simulated lung fluid, interactions with alveolar macrophage, and safety after aerosolization of the particles into the lungs. In addition, we monitored the pharmacokinetics of the optimized formulation in healthy animals and studied the vasodilatory effects of the formulations in an animal model of PAH.

2. Materials and methods

2.1. Materials

PLGA polymers (inherent viscosity 0.55–0.75 dL/g) were purchased from Lactel Absorbable Polymers (Birmingham, AL) and sildenafil citrate from Biotang Inc. (Lexington, MA). Male Sprague–Dawley (SD) rats (250–350 g) were supplied by Charles River Laboratories (Wilmington, MA), and for fluorescent particles, DiD oil (1,1′-Dioctadecyl-3,3,3′,3′ tetramethylindodicarbocyanine perchlorate) was purchased from Thermo Fisher Scientific Inc. (Waltham, MA). Rat PASMC cells were from Dr. Eva Nozik-Grayck's laboratory at the University of Colorado. All other chemicals were HPLC grade and purchased from Sigma-Aldrich (St. Louis, MO). All animal studies were performed in compliance with the NIH Guideline for the Care and Use of Laboratory Animals under an approved protocol (AM-10012).

2.2. Methods

2.2.1. Preparation of microparticles

Sildenafil loaded microparticles were prepared by a water-in-oil-in-water ($W_1/O/W_2$) double emulsion–solvent evaporation method after slight modification of our previously established method [21]. Briefly, 0.5 mL of an internal aqueous phase (IAP, W_1) containing sildenafil citrate (20 mg/mL) in methanol-water (20:80) was first emulsified in dichloromethane (organic phase, OP) containing 250 mg of polymer using a Branson Sonifier 450 (Branson Ultrasonics Corporation, Danbury, CT), in the absence or presence of 0.5% or 1.25% polyethyleneimine (PEI) in water. A double emulsion was prepared by homogenizing primary (W_1/O) emulsion with 0.5% w/v polyvinyl alcohol (PVA) solution (EAP, W_2). The organic phase was removed and particles were hardened by stirring the resulting double emulsion for 8 h at room temperature. The particles were then washed thrice with water and lyophilized for 48 h to get free-flowing powdered formulations. Each formulation was prepared in triplicate and stored at 4 °C for further studies. Similarly, fluorescent particles were prepared by adding DiD oil in the OP.

2.2.2. Physical characterization of sildenafil microparticles

Sildenafil loaded microparticles were characterized for their morphology, size, aerodynamic diameter, and zeta potential. The morphology of the microparticles was examined in a Hitachi S-4300 (Freehold, NJ) scanning electron microscope (SEM). The volume-based mean diameter (D_v) and particle size distribution of the formulations were measured in a Malvern® Mastersizer 2000 (Malvern Instruments Ltd., Worcestershire, UK) particle size analyzer. To measure D_v, freeze-dried particles (~15 mg) were dispersed in deionized water by a Hydro 2000MU sample-dispersion unit and then pumped into the particle size analyzer. The zeta potential was determined in a Nano ZS90 Zetasizer (Malvern® Instruments Ltd., Worcestershire, UK) after dispersing the particles in 1 × PBS buffer. The mass median aerodynamic diameters (MMAD) were measured in an eight-stage Marc-II Andersen Cascade Impactor (Westech Instruments Inc., Marietta, GA). Preweighed glass fiber filter papers were placed on the plates of each stage, and solid freeze-dried particles were filled in a size 4 gelatin capsule (Capsuline Inc., Pompano Beach, FL). The particles were fired into the impactor from a HandiHaler® (Pfizer, Brooklyn, NY) at a flow rate of 28.3 L/min, and the particles deposited on each stage were weighed after deducting the weights of glass fiber filter papers without the powder. Each sample was run into the impactor thrice, percent cumulative weight of the particles was plotted, on a semi-log graph paper, against the effective cut-off diameter (μm) of various stages, and the MMAD was recorded from 50% of the % cumulative weight scale.

2.2.3. Entrapment efficiency

The entrapment efficiency of the formulations was determined directly by extracting the drug from PLGA particles. Particles (~5 mg) were dissolved in a 70:30 mixture of methanol:dimethyl sulfoxide and the absorbance of the extracted drug was measured at 311 nm in a spectrophotometer (Hewlett Packard, Palo Alto, CA). The concentration of sildenafil, extracted from the particles, was read from a calibration curve of absorbance versus concentrations of sildenafil citrate in the vehicle.

2.2.4. Assay of sildenafil in the simulated lung fluid (SLF) and rat plasma

By adding 0.1% Tween 80 at pH 7.4 to the Moss formula [22] and stirring the solution at 300 rpm at 37 ± 1 °C (Table 2), we prepared an SLF [23]. We periodically sampled SLF, diluted with the mobile phase, centrifuged at 17,000 g for 15 min, and measured the concentration of sildenafil in SLF by an ultra-high performance liquid chromatographic (UPLC) method. For chromatographic separation, we used a Kinetex® C18 UHPLC column (50 × 2.1 mm, 1.3 μm; Phenomenex, Torrance, CA), gradient elution of the mobile phase comprising 1% formic acid in water and 0.1% formic acid in methanol, and a flow rate of 0.25 mL/min. Rosiglitazone maleate, at a concentration of 200 μg/mL, was used as the internal standard.

We have determined the concentration of sildenafil in rat plasma in an AB SCIEX QTRAP® 5500 tandem mass spectrometer (Framingham, MA) attached to a UPLC system and an electrospray ionization (ESI) interface. Using a protein precipitation technique with methanol, we extracted sildenafil from the plasma. For internal standard, we used deuterated sildenafil. Keeping the chromatographic conditions just as described above, we maintained the ESI source in a positive ionization mode, and quantified the drug by means of multiple-reaction-monitoring (MRM) method with the transitions of the parent ions to the product ions of m/z 475.3 → 283.2 for sildenafil and m/z 483.4 → 283.3 for deuterated sildenafil, respectively.

2.2.5. In-vitro release profiles of sildenafil

To study the release of sildenafil from the microparticles, ~10 mg freeze-dried particles were suspended in 1 mL of SLF. An aliquot of samples (100 μL) was withdrawn over a period of 36 h at various time intervals, centrifuged and the drug concentration in the samples was quantified by the UPLC method described above.

Table 1
Compositions of different formulations of PLGA-based particles of sildenafil.

Formulation	Polymer	IAP	EAP
S1	PLGA 50/50	Water	0.5% PVA
S2	PLGA 50/50	0.5% PEI	0.5% PVA
S3	PLGA 50/50	1.25% PEI	0.5% PVA
S4	PLGA 75/25	Water	0.5% PVA
S5	PLGA 75/25	0.5% PEI	0.5% PVA
S6	PLGA 75/25	1.25% PEI	0.5% PVA