Published in final edited form as:

Pharm Res. 2016 March ; 33(3): 563–572. doi:10.1007/s11095-015-1808-x.

Sustained Pulmonary Delivery of a Water-Soluble Antibiotic Without Encapsulating Carriers

Winston Ong¹, Pawel Nowak¹, Yen Cu¹, Lisa Schopf¹, James Bourass¹, Elizabeth Enlow¹, Samuel M. Moskowitz², and Hongming Chen¹

¹ Kala Pharmaceuticals, Inc., 100 Beaver Street, Suite 201 Waltham, Massachusetts 02453, USA

² Department of Pediatrics, Massachusetts General Hospital and Harvard Medical School, Boston, Massachusetts 02114, USA

Abstract

Purpose—Traditional polymeric nanoparticle formulations for prolonged local action during inhalation therapy are highly susceptible to muco-ciliary clearance. In addition, polymeric carriers are typically administered in high doses due to finite drug loading. For toxicological reasons, these carriers and their degradation byproducts are undesirable for inhalation therapy, particularly for chronic use, due to potential lung accumulation.

Methods—We synthesized a novel, insoluble prodrug (**MRPD**) of a time-dependent β -lactam, meropenem, and formulated **MRPD** into mucus-penetrating crystals (**MRPD-MPCs**). After characterizing their mucus mobility (*in vitro*) and stability, we evaluated the lung pharmacokinetics of intratracheally instilled **MRPD-MPCs** and a meropenem solution in guinea pigs.

Results—Meropenem levels rapidly declined in the lungs of guinea pigs receiving meropenem solution compared to those given **MRPD-MPCs**. At 9 h after dosing, drug levels in the lungs of animals that received meropenem solution dropped to 12 ng/mL, whereas those that received **MRPD-MPCs** maintained an average drug level of 1,065 ng/mL over a 12-h period.

Conclusions—This work demonstrated that the combination of prodrug chemistry and mucus-penetrating platform created nanoparticles that produced sustained levels of meropenem in guinea pig lungs. This strategy represents a novel approach for sustained local drug delivery to the lung without using encapsulating matrices.

Keywords

antibiotics; drug delivery; meropenem; mucus; nanoparticle; prodrug

Winston Ong winston.ong@kalarx.com.

Electronic supplementary material The online version of this article (doi:10.1007/s11095-015-1808-x) contains supplementary material, which is available to authorized users.

INTRODUCTION

Pulmonary delivery of small-molecule, water-soluble drugs (<1,000 Da) for prolonged local action in the lung epithelia and airway mucosa is very challenging [1, 2]. When delivered as free drugs, they are expected to rapidly dissolve in mucus and are therefore not highly susceptible to muco-ciliary clearance. However, dissolved small-molecule drugs are likely to have short residence time in the lung due to immediate absorption into systemic circulation through the lung epithelia. Therefore, in order to maximize local drug duration, an inhaled formulation needs to effectively slow down systemic absorption.

Encapsulation of drugs inside particle carriers, such as polymeric and solid lipid particles, liposomes and micelles, is the conventional strategy to formulate sustained-releasing inhalable drugs [1]. However, encapsulating carriers bear practical limitations when used for inhalable therapeutics, especially for chronic indications, due to the potential pulmonary toxicity arising from the accumulation of these encapsulating agents and their breakdown products in the lung [3, 4]. Several studies even support a correlation between morbidity and mortality with inhalation of non-therapeutic nanoparticles [5, 6]. Given these long-term safety concerns, dosage forms containing minimal levels of encapsulating agents are preferable for inhalable therapeutics.

In addition to safety concerns, muco-ciliary clearance is another barrier to overcome for pulmonary delivery of encapsulated drugs. Muco-ciliary clearance is believed to be predominantly manifested in the luminal mucus layer (LML) wherein the majority of the inhaled particulates are trapped and removed by mucus to protect the underlying respiratory epithelia from exposure to foreign matter [7]. As the LML is freshly secreted and continuously swept by the cilia out of the lungs and into the trachea in the order of minutes, a majority of the inhaled drugs that are still encapsulated are eliminated, resulting in poor drug availability [2, 8].

Drugs encapsulated in polymeric mucus-penetrating particles (MPPs) have shown prolonged exposure in mucosal tissues compared to non-MPPs [9–11]. It is believed that the dense coating of methoxy poly(ethylene) glycol (mPEG) stabilizers present in MPPs allowed the nanoparticles to efficiently bypass mucus clearance mechanisms. In order to overcome the potential problems resulting from the aforementioned safety concerns of encapsulating carriers, we engineered an entirely novel delivery platform, mucus-penetrating crystals (MPCs), that could be applied to various types of local therapies [12, 13]. Unlike polymeric MPPs, the inner core of the MPCs described herein are comprised entirely of unencapsulated crystalline drug. Although the MPCs described herein include surface-adsorbed surfactants, the particle core of the MPCs is excipient-free. In effect, the excipient loading in an MPC dosage is substantially lower relative to conventional drug-encapsulated formulations, making these MPCs ideally suited for inhalation therapies.

Sustained exposure is particularly important for drugs that exhibit time-dependent pharmacodynamics, such as β -lactam antibiotics. We demonstrate herein MPC formulations that can produce prolonged duration of meropenem in the lungs. We especially selected this highly soluble antibiotic as the model compound because drugs exhibiting fast dissolution

are extremely difficult to deliver in a sustained manner without using rate-controlling matrices to regulate drug release. To provide controlled dissolution from MPC, we first synthesized a novel lipophilic prodrug of meropenem (**MRPD**). The prodrug was then formulated into MPCs (**MRPD-MPC**) to impart muco-inert properties on the nanoparticles. Upon deposition in the conducting airway, we hypothesized that **MRPD-MPC** would overcome muco-ciliary clearance to remain in the lung, slowly dissolve to produce steady levels of the prodrug and hydrolyze to release the pharmacologically active meropenem. To test this hypothesis, we evaluated the lung pharmacokinetics (PK) of intratracheally-instilled **MRPD-MPC** suspension and meropenem solution in guinea pigs.

MATERIALS AND METHODS

Synthesis of MRPD

The novel prodrug **MRPD** and its precursors were synthesized as described below.

Preparation of (benzoyloxy)methyl-4-nitrophenyl carbonate (1)—Iodomethyl- 4-nitrophenyl carbonate (0.97 g, 3.0 mmol) was dissolved in toluene (20 mL). Benzoic acid (0.55 g, 4.5 mmol) and silver oxide (1.24 g, 5.36 mmol) were added. The resulting mixture was heated at 80°C for 2 h and filtered through silica pad with the aid of more toluene. Volatiles were evaporated under reduced pressure, yielding compound **1** as a yellow oil (0.89 g, 93%), which was used further without purification.

Preparation of chloromethyl benzoate (2)—Sodium benzoate (4.8 g 33.3 mmol), sodium bicarbonate (8.4 g 100.0 mmol) and tetrabutylammonium sulfate (1.1 g, 3.3 mmol) were dissolved in water (70 mL). Dichloromethane (70 mL) was added followed by chloromethyl chlorosulfonate (4.2 mL, 40.3 mmol). The resulting mixture was a biphasic solution that was vigorously stirred for 3 h. The phases were separated. The organic phase was washed with water (2×50 mL) and dried over anhydrous magnesium sulfate. The solution was filtered through a small plug of silica (5 g) and evaporated under reduced pressure to yield compound **2** as a colorless liquid (5.2 g, 92%). The analytical data for compound **2** were identical to those reported in literature [14].

Preparation of iodomethyl benzoate (3)—Compound **3** was prepared by a modification of a literature procedure [15]. Chloromethyl benzoate (2.7 g, 15.9 mmol) was dissolved in acetone (20 mL). Sodium iodide (7.1 g, 47.6 mmol) was added, and the resulting mixture was stirred for 3 h at 45°C, diluted with acetone (100 mL), filtered in the absence of light, and evaporated under reduced pressure. The residue was dissolved in diethyl ether (100 mL), washed with aqueous sodium bicarbonate and aqueous sodium thiosulfate, dried over anhydrous magnesium sulfate, and evaporated under reduced pressure in the absence of light to yield compound **3** as a yellow oil (3.3 g, 79%). The analytical results of compound **3** were identical to literature data [15]. Compound **3** was used immediately in the subsequent step.

Preparation of (4R, 5S, 6S)-3-[[[(3S,5S)-5-[(dimethylamino)-carbonyl]-1-[[benzyloxymethoxy]carbonyl]-3-pyrrolidinyl]thio]-6-[(1R)-1-hydroxyethyl]-4-

methyl-7-oxo-1-azabicyclo[3.2.0]-hept-2-ene-2-carboxylic acid

benzyloxymethyl ester (MRPD)—Meropenem trihydrate (1.27 g, 2.90 mmol) was dissolved in dimethylformamide (20 mL). (Benzoyloxy)methyl-4-nitrophenyl carbonate (compound **1**, 0.92 g, 2.90 mmol) was added as a solution in dimethylformamide (2 mL). The resulting mixture was stirred for 1 h. Anhydrous sodium carbonate (0.62 g, 5.80 mmol) and iodomethyl benzoate (compound **3**, 1.52 g, 5.80 mmol) were added. The reaction suspension was stirred for 1 h, then ethyl acetate (200 mL) was added and the resulting mixture was washed in sequence with water (50 mL) and aqueous saturated sodium bicarbonate (3×50 mL), then dried over anhydrous magnesium sulfate. The volatile organic solvents were evaporated under reduced pressure and the resulting residue was dissolved in dichloromethane (100 mL) and further evaporated under reduced pressure. The resulting thick oil was dissolved in minimal amount of dichloromethane (about 5 mL) and poured into diethyl ether (200 mL) to precipitate the semi-solid crude product, which was filtered and dissolved in a mixture of ethyl acetate and acetone (4:1, 20 mL). The resulting solution was filtered through a silica pad (10 g) with the aid of additional solvent mixture (ethyl acetate and acetone (4:1)) as needed. Volatile solvents were evaporated under reduced pressure to yield a yellow solid (1.45 g), which was purified by preparative HPLC (Zorbax C18, 50 mm×250 mm) running on a gradient from 50:50 to 10:90 water (containing 0.1% formic acid)/acetonitrile (containing 0.1% formic acid) in 10 min at a flow rate of 118 mL/min. The relevant fractions were neutralized with saturated sodium bicarbonate solution (5 mL), and volatile solvents were evaporated under reduced pressure to yield a cloudy suspension that was extracted with dichloromethane. The organic layer was dried over anhydrous magnesium sulfate and evaporated under reduced pressure to dryness. The resulting residue was dried *in vacuo* to yield **MRPD** as a white solid (280 mg, 14%). ¹H NMR (CDCl₃): δ 8.04 (4H, m), 7.55 (2H, m), 7.42 (4H, m), 6.12 (1H, d), 6.08 (1H, d), 6.02 (1H, d), 5.89 (1H, d), 4.70 (1H, m), 4.21 (1H, m), 4.16 (1H, m), 4.09 (1H, m), 3.62 (1H, m), 3.42 (1H, m), 3.36 (1H, m), 3.18 (1H, m), 3.08 (3H, s), 3.02 (3H, s), 2.96 (3H, s), 2.81 (3H, s), 2.67 (1H, m), 1.88 (1H, m), 1.28 (3H, d), 1.12 (3H, d) ppm. ¹³C NMR (CDCl₃): δ 172.95, 172.87, 170.86, 170.65, 165.69, 165.63, 165.24, 159.65, 159.59, 152.65, 152.13, 150.98, 150.64, 133.92, 133.86, 130.33, 130.30, 130.23, 129.27, 129.25, 129.13, 128.75, 128.73, 128.71, 125.11, 125.04, 81.04, 80.42, 80.32, 80.30, 66.15, 66.12, 60.23, 60.16, 56.57, 56.47, 56.28, 55.90, 54.82, 54.46, 44.35, 40.95, 40.25, 37.25, 37.10, 36.40, 36.22, 35.93, 35.32, 22.00, 21.96, 17.29 ppm. LC-MS: *m/z* (M⁺) calculated 695.7, found 696.2. **MRPD** crystallized from methanol/water (2:1, 5 mg/mL total concentration) or ethyl acetate/methyl *t*-butyl ether (1:1, 40 mg/mL total concentration) yielded crystals that were suitable for wet milling.

Solubility of MRPD in Water

An Agilent 1100 Series high-performance liquid chromatography (HPLC) equipped with a diode array detector and a Waters Xterra MS C₁₈ column (3.5 μm particles, 3.0×150 mm) was used to evaluate the solubility of neat **MRPD** in water. The composition of the eluting mobile phase ramped linearly from 98:2 to 0:100 of 0.1% phosphoric acid in water:acetonitrile over 10 min. The injection volume, elution flow rate, column temperature and monitoring wavelength were 5 μL, 0.6 mL/min, 40°C and 323 nm, respectively.

Briefly, neat **MRPD** (1 mg) was suspended in triply-distilled, deionized water (1 mL), then gently stirred at ambient temperature for 24 h. Then, the suspension was transferred into a 1.5-mL tube and spun at 13,200 rpm using an Eppendorf 5415 D centrifuge. The supernatant was carefully transferred into a sampling vial for HPLC content analysis of the prodrug. The corresponding area-under-the-curve of **MRPD** in the chromatogram was approximately 50% compared to that of a 5 µg/mL standard in acetonitrile.

Formulation of MRPD-MPC

Mucus-penetrating crystals were prepared using methods of milling crystals described elsewhere [16]. Briefly, a phosphate-buffered saline slurry containing 5% **MRPD** (>10 µm diameter) and 5% poloxamer 407, which acts as surface stabilizer, was added to an equal bulk volume of 1-mm Ceria-stabilized zirconium oxide beads (Next Advance, Inc., New York) in a glass vial. A magnetic stirring bar was used to agitate the beads, stirring at approximately 500 rpm. After 4 days of milling, the **MRPD-MPCs** reached the desired diameter and polydispersity index (PDI) of <500 nm and <0.2, respectively. Particle size of freshly-prepared and stored particles (i.e., as described in stability studies section herein) was assessed using dynamic light scattering (DLS) as follows: the suspension of **MRPD** was diluted to 0.01–0.05% w/v using 0.7% w/v sodium chloride, then particle size was measured using Zetasizer Nano ZS90 (Malvern, United Kingdom). The Z-average diameter and PDI were reported as an average of three separate measurements.

Stability Studies

Physical Stability—A suspension of freshly-prepared **MRPD-MPC** was stored in a clear scintillation vial under ambient conditions. Aliquots were taken at 1-, 2-, 4- and 14-week time points, then particle size was determined as described (vide supra).

Chemical Stability—An HPLC method similar to the solubility study was used to evaluate the chemical stability of **MRPD-MPC**, except that a Waters SunFire C₁₈ column (3.5 µm particles, 4.6×150 mm) was used and the mobile phase elution ramp time and flow rate were adjusted to 8 min and 1.2 mL/min, respectively. A triplicate of diluted solutions of **MRPD**, typically around 0.3–0.4 mg/mL, were prepared by dissolving an appropriate aliquot volume of **MRPD-MPC** suspension in 90% ethanol in separate vials, then the prodrug content in each solution was determined by HPLC. The peak area of the prodrug in a freshly-prepared **MRPD-MPC** suspension was assigned as 100% content. The peak areas of **MRPD** at the 1-, 2- and 4-week time points were reported relative to the values obtained for the freshly-prepared **MRPD-MPC** suspension.

Particle Tracking of MRPD-MPC in Cervicovaginal Mucus (CVM)

Human CVM was collected (Boston IVF, MA) following a protocol approved by the New England Institutional Review Board. Samples for particle tracking were prepared for microscopy by adding 1 µL of 5% w/v **MRPD-MPC**, which is weakly blue fluorescent, to 20 µL of reconstituted CVM along with positive- and negative-control particles. Green fluorescent, carboxylate-modified 200-nm polystyrene nanoparticles (Life Technologies, NY) were used as a negative control to confirm the barrier properties of CVM. Red

fluorescent, 200-nm PS particles covalently modified with mPEG-5000 were used as a positive control due to its well-established mucus penetration behavior. Using a fluorescent microscope equipped with a CCD camera, movies approximately 15 s were captured at a temporal resolution of 66.7 ms (15 frames/s) under 100× magnification for each type of particle: **MRPD-MPC**, negative control, and positive control. At minimum, two sets of measurements from different regions in the CVM were acquired for each particle type. Individual trajectories of multiple particles ($n = 14$) were then measured using Image-Pro Analyzer v. 7.0 (Media Cybernetics, Inc., MD) over a timescale of at least 2.0 s (30 frames). The resulting mobility data are quantified in Supplementary Table S1 as ensemble-averaged velocity, V_{mean} , which is the average particle velocity of the ensemble in the measured regions during the specified timescale.

Scanning Electron Microscopy (SEM)

Images were obtained using a Phenom Desktop SEM (Phenom-World, The Netherlands). **MRPD-MPC** particles (0.5 mg) were added to water (1 mL) and centrifuged at 12, 800 rpm for 10 min. The supernatant was removed, then the pellet was deposited onto an SEM sample holder covered with carbon tape. After drying under ambient conditions, sample was coated using a Quorum SC7620 Sputter Coater (Quorum Technologies Ltd, United Kingdom) with a gold-palladium target. Images were collected at high resolution with the detector set to full topography.

In Vitro Metabolism of MRPD in Human Lung Microsomes

Preparation of Test Solutions—The test article was **MRPD** while meropenem served as a control article. Fresh 5-mM stock solutions of test and control articles were prepared separately in DMSO and further diluted to 1.25 mM with acetonitrile. These stock solutions were further diluted and incubated with lung microsomes as described below.

Metabolic Stability Assay—Pooled lung microsomes (Xenotech, Kansas) at 10 mg/mL were quick-thawed in a 37°C water bath and diluted fivefold in 100 mM potassium phosphate buffer, pH 7.4 (warmed to 37°C) to a concentration of 2 mg/mL. The test and control articles were diluted 125-fold from the 1.25 mM intermediate stock solutions in 100 mM potassium phosphate, pH 7.4, 4 mM NADPH (Sigma-Aldrich) (target of 10 μ M test article). Two hundred microliters of this 10- μ M compound solution (containing either the test or control compound and NADPH cofactor) was added to an equal volume of 2 mg/mL lung microsomes in polypropylene 96-well plates to initiate the incubation. The final assay concentrations were 5 μ M compound, 1 mg/mL lung microsomes and 2 mM NADPH. Immediately after adding compounds to the microsomes, triplicate 50- μ L aliquots were removed (for T_0 samples) and then quenched with 200 μ L of the acetonitrile quenching solution to precipitate protein. The quenching solution was acetonitrile containing 250 ng/mL of carbutamide and glyburide as internal standards. The assay incubation plate was maintained at 37°C with gentle agitation in a 37°C rocking incubator. Aliquots were similarly removed and quenched after 15, 30, 60, 90 and 120 min. The meropenem control sample was quenched at T_0 (i.e., this T_0 signal was used as a 5- μ M reference point in order to calculate relative % conversion). Quenched samples were maintained on ice before they were centrifuged at 2,000×g for 5 min at 5°C in a Thermo Sorvall Legend refrigerated

centrifuge. Fifty-microliter aliquots of the supernatants were removed and diluted with 100 μ L water to reduce the organic content prior to analysis by LC/MS/MS.

Analysis—An ACE C₁₈, 3 μ m, 50 $\times$ 2.1 mm column with a linear gradient (0.5 mL/min flow rate) from 100% mobile phase A (0.1% formic acid in water) to 95% mobile phase B (0.1% formic acid in acetonitrile) was used. The injection volume was 20 μ L. Electrospray in positive mode was used. Measurements were made with an ABI Sciex 4000 LC/MS/MS instrument. Data were captured and processed using Analyst v.1.4.2. The MRM transitions used were as follows: meropenem=384.3/114.0, carbutamide=272.2/156.0 and glyburide=494.2/369.0.

Pharmacokinetic Profiling in Guinea Pigs

In-Life Study—The study was conducted at Charles River Laboratories Montreal, Canada. Fifty three male guinea pigs (*Cavia porcellus*) approximately 6 weeks old (Charles River, Canada) were used. Animals were given a single dose of either 8.0-mg/mL solution of meropenem or 14.5-mg/mL suspension of **MRPD-MPC** (equivalent to 8.0 mg/mL of meropenem) using an intratracheal microsyringe (PennCentury Wyndmoor, PA). The dose volume was 0.2 mL per animal. At the designated time points (0.083, 0.25, 0.5, 1, 3, 6, 9 and 12 h) lungs were removed, snap-frozen and shipped to a bioanalytical laboratory (Nextcea, MA) for drug measurements.

Analysis—A Varian Metasil C18 50 $\times$ 2.0-mm column with a linear gradient (0.4 mL/min flow rate) from 100% mobile phase A (0.1% formic acid in water) to 95% mobile phase B (0.1% formic acid in acetonitrile) was used. The injection volume was 10 μ L. Frozen tissues were stored at -80°C before being homogenized 1:3 w/v with acetonitrile-water (1:1 v/v). Cold acetonitrile was added to the resulting supernatant (4:1 v/v) to precipitate proteins prior to analysis. Electrospray in positive mode was used, and verapamil was used as the internal standard. Measurements were made with an ABI Sciex 5000 LC/MS/MS instrument. Data were captured and processed using Analyst v.1.4.2. The MRM transitions used were as follows: meropenem=384.3/340.2, **MRPD**=696.2/544.2 and verapamil=455.4/165.1.

RESULTS

Preparation of MRPD-MPC

A prerequisite for formulating meropenem as MPCs was to synthesize a hydrophobic form of this otherwise hydrophilic drug. This was accomplished by chemically masking the hydrophilic carboxylic acid and amine functional groups of meropenem with hydrophobic benzoate esters and a formaldehyde bridge (Scheme 1). The resulting novel prodrug (**MRPD**) was isolated as a crystalline compound, as evidenced by polarized light microscopy (Fig. 1), and was found to be markedly more hydrophobic than meropenem, as indicated by over a thousand-fold decrease in its solubility (*S*) in water (*S* < 10 μ g/mL) compared to the parent drug (*S* > 50 mg/mL) [18]. Coarse crystals of **MRPD** (diameter > 10 μ m) were then milled in an aqueous suspension containing poloxamer 407 and milling media. SEM confirmed that the coarse irregular crystals were reduced to fine crystalline nanoparticles of a narrow size distribution. The Z-average diameter of these particles

measured by DLS was 443 nm with a PDI of 0.173 (Fig. 1). During storage, the aqueous **MRPD-MPC** suspensions appeared physically and chemically stable (Supplementary Figs. S1 and S2) for 14 and 4 weeks, respectively. Examination of physical and chemical stability was not conducted at longer time points.

Enhanced Mobility of MRPD in Human Mucus

MRPD is weakly fluorescent due to the phenyl moieties present in the protecting groups. Thus, mobility of **MRPD-MPCs** in human mucus was confirmed *ex vivo* using a tracking method that measures real-time displacement of multiple particles via fluorescent microscopy [19, 20]. Control nanoparticles comprised of a 200 nm polystyrene (PS) core terminated with either mPEG-5000 (positive control) or carboxylate (negative control) units were used to benchmark the mobility of **MRPD-MPCs** [17, 19, 20]. Table I shows that the recorded mean ensemble velocity (V_{mean}) of the positive control was $6.6 \pm 0.6 \mu\text{m/s}$. Under the same conditions, the V_{mean} of the negative-control particles was approximately an order of magnitude lower ($V_{\text{mean}} = 0.58 \pm 0.80 \mu\text{m/s}$). The mobility of **MRPD-MPCs** was approximately half of the positive control ($V_{\text{mean}} = 3.2 \pm 1.1 \mu\text{m/s}$) but significantly higher than the negative control. The mobility of **MRPD-MPCs** can be further visualized using real-time particle tracking to determine particle displacement over time (Supplementary Fig. S3). Within the 15-s data acquisition period, the positive-control and **MRPD-MPCs** underwent ensemble-average maximum displacements of $6.0 \pm 2.8 \mu\text{m}$ and $2.7 \pm 1.8 \mu\text{m}$ from origin, respectively. Under the same timeframe, the negative control nanoparticles were significantly hindered, as evidenced by an average displacement of only $0.29 \pm 0.85 \mu\text{m}$. Clearly, both the V_{mean} and particle displacement results indicate that **MRPD-MPCs** exhibit good mobility in human mucus.

In Vitro Metabolism of MRPD

The efficiency at which **MRPD** undergoes metabolic conversion to release meropenem was evaluated in human lung microsomes (HLMs) [21]. Homogenized HLMs were incubated with pre-dissolved **MRPD** and tandem mass spectrometry was used to quantify the amount of liberated meropenem. Results in Fig. 2 show that **MRPD** hydrolyzed into meropenem within minutes of incubation and hydrolysis reached near completion (98%) after 90 mins. Little or no meropenem was detected when the incubation assay was performed in the absence of HLMs, suggesting that prodrug hydrolysis was metabolically driven.

Lung Pharmacokinetic (PK) Studies of MRPD

As a carbapenem, meropenem undergoes rapid hydrolysis and inactivation by dehydropeptidase I, a ring-opening β -lactamase. This enzyme is more active in mice, rats, rabbits and monkeys than in humans, beagle dogs and guinea pigs [22, 23]. Therefore, guinea pigs were chosen to evaluate lung PK of **MRPD**. Intratracheal administration was used to maximize delivery of the dose to the conducting airways. Stoichiometric equivalent doses of **MRPD** and meropenem at 2.9 and 1.6 mg, respectively, were administered to the animals. The dose of meropenem was chosen based on the daily dosage of aztreonam (Cayston®), a monobactam that is currently the only approved inhaled β -lactam antibiotic [24]. The standard human dose of 75 mg aztreonam thrice-daily (225 mg/day), or 3.75

mg/kg each day (assuming a 60 kg human), equates to a weight-adjusted dose of approximately 1.6 mg meropenem for a 425-g guinea pig.

Peak concentrations ($C_{\max}$) of meropenem were observed in the whole lung within minutes after intratracheal administration in animals receiving either meropenem solution or **MRPD-MPCs**. However, meropenem levels rapidly declined in animals receiving the solution formulation compared to those given **MRPD-MPCs**. At 9 h after dosing, meropenem levels in the solution group dropped to 12 ng/mL (Fig. 3 and Supplementary Table S1). In contrast, animals receiving **MRPD-MPCs** maintained an average lung meropenem level of at least 1,065 ng/mL over a 12-h period and were statistically higher at 6, 9 and 12 h post-dose when compared to animals dosed with meropenem solution. Concentrations of intact **MRPD** were also elevated throughout the 12-h examination period. At 12 h post-dose, lung tissue homogenates still contained over 20,000 ng/mL of the intact prodrug.

DISCUSSION

Our main goal was to identify a pharmaceutically acceptable formulation that can deliver a water-soluble, crystalline compound to the lung in a sustained manner without using rate-controlling carriers. Meropenem in its native form is highly water soluble ($S > 50$ mg/mL), making it incompatible for sustained release. We designed and synthesized a lipophilic meropenem prodrug, **MRPD**, with drastically reduced solubility ($S < 10$ µg/mL) and then formulated this crystalline compound into **MRPD-MPCs** to introduce muco-inert properties. Despite the known chemical instability of the β -lactam moiety in solution [18, 25, 26], the aqueous suspensions of **MRPD-MPCs** were chemically stable for at least 4 weeks at ambient temperature (Supplementary Fig. S1), likely due to the very low solubility of **MRPD**. The core of each **MRPD-MPC** particle is comprised exclusively of the crystalline prodrug and devoid of rate-controlling carriers. We hypothesize that a slow-dissolving prodrug particle is sufficient to regulate the production of solubilized **MRPD**, which will then steadily metabolize into meropenem *in vivo*.

A key requirement for producing **MRPD-MPCs** is to reduce the diameter of **MRPD** coarse crystals to < 500 nm. This upper limit in particle dimension was indicated previously as being necessary to avoid adhesive interactions of particles with mucin fibers [8]. With wet milling, we produced **MRPD-MPC** that was 443 nm in diameter and subsequently confirmed by individual particle tracking analysis that these nanoparticles were mobile in mucus (Table I and Supplementary Fig. S3). A previous study using MPPs estimated that this diffusivity would result in the transport of approximately 35% of dosed MPPs across a 30-µm thick LML within 10 min [20]. Given that LML renews every 4–15 min [8], both the reported MPP diffusivity and our particle tracking data provided an early indication that, in principle, a considerable fraction of **MRPD-MPCs** should resist mucociliary clearance following deposition into the conducting airways of guinea pigs.

The mobility of **MRPD-MPCs** measured in *ex vivo* mucus appears to have translated *in vivo*, as sustained lung concentrations of **MRPD** at $> 5,000$ ng/mL were observed for at least 12 h. The sustained drug PK behavior is presumed to result from the efficient penetration of **MRPD-MPCs** through the LML (Fig. 4, a-I) and accumulation into the PCL (Fig. 4, a-II),

thereby effectively avoiding muco-ciliary clearance. As particles of **MRPD-MPC** slowly dissolved (Fig. 4, a-III), a portion of the solubilized **MRPD** was absorbed into the systemic circulation as intact prodrug, while the rest were metabolized into meropenem (Fig. 4, a-IV). Over time, the continuous dissolution and hydrolysis of **MRPD** into meropenem provided steady local drug concentrations of >1,000 ng/mL. At 12 h post-dose, the concentration of **MRPD** remained elevated, suggesting that meropenem could be sustained at >1,000 ng/mL for an even longer duration. In contrast, the control meropenem solution lacked a mechanism to extend drug duration. Once dosed, the drug was eliminated through metabolism and systemic absorption without being “replenished”. Even though the C_{max} of meropenem in the solution group exceeded 70,000 ng/mL at 5 min post-dose, drug concentration declined below 1,000 ng/mL at 3 h post-dose until reaching only 10 ng/mL at 12 h post-dose.

The *in vitro* metabolic data of **MRPD** in HLMs suggest that the dissolved prodrug should enzymatically hydrolyze *in vivo* to rapidly liberate meropenem. This prediction is consistent with the observation that lung concentrations of meropenem after **MRPD-MPC** dosing were sustained over 12 h, and implies that hydrolysis was not rate-limiting *in vivo*. The concentration of meropenem from the **MRPD-MPC** group at 12 h post-dose was >100-fold higher in the lung compared to the meropenem solution group (1,470 ng/mL vs. 10 ng/mL). This observation is remarkable because prolonged lung exposure of a soluble drug was accomplished with the novel combination of prodrug chemistry and the mucus-penetrating technology without drug encapsulation in a carrier matrix, which is typically required to achieve sustained local drug delivery [27].

The data described herein also shows the potential to develop **MRPD-MPCs** as an inhalable antibiotic to treat cystic fibrosis (CF) lung infections caused by *P. aeruginosa*, the most common bacterial pathogen that causes chronic endobronchial infection among CF patients [28–30]. Sustained delivery of meropenem is highly desirable from a drug development perspective due to its time-dependent pharmacodynamics [31]. An inhalable dosage form of this antibiotic will be particularly beneficial to CF patients since intravenous meropenem is the current frontline therapy to treat CF lung infections [28]. At the dose used in the guinea pig study, the meropenem concentration at 12 h post-dose was marginally lower than the MIC_{90} of the antibiotic against *P. aeruginosa* (8,000 ng/mL) [28]. It is conceivable that concentrations above MIC_{90} can be achieved by simply increasing the dose of **MRPD-MPCs**. Additional experimentation should also be conducted to determine whether elevated concentrations can be achieved for 24 h to enable the development of a once-daily inhalable meropenem. If successful, this would represent a significant improvement over Cayston®, which is currently the only approved inhaled β -lactam and is indicated thrice-daily [32, 33], likely due to its relatively short half-life in sputum [34].

CONCLUSION

This work has demonstrated that a fundamentally novel combination of prodrug chemistry and the mucus penetrating platform can produce sustained levels of a soluble drug, meropenem, in guinea pig lungs. This strategy represents a novel platform for sustained drug delivery locally to the lung without the need of encapsulating matrices. Finally, the results

show the potential of **MRPD-MPC** to be developed as an inhalable therapeutic to treat local diseases, such as CF lung infections, warranting further development activities, such as formulation of a dry powder, optimizing powder flowability, conducting more rigorous stability testing, etc.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGMENTS AND DISCLOSURES

The work described was partially supported by Award Number R43HL106899 from the National Heart, Lung and Blood Institute. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Heart, Lung and Blood Institute or the National Institutes of Health.

ABBREVIATIONS

CF	Cystic fibrosis
C_{max}	Peak concentration
CVM	Cervicovaginal mucus
HLMs	Human lung microsomes
HPLC	High-performance liquid chromatography
LML	Luminal mucus layer
MIC	Minimum inhibitory concentration
MPC	Mucus-penetrating crystal
mPEG	Methoxy poly(ethylene) glycol
MPP	Mucus-penetrating particle
MRPD	Meropenem prodrug
MRPD-MPC	MRPD formulated as a mucus-penetrating crystal
n	Particle population
PCL	Periciliary layer
PDI	Polydispersity index
PK	Pharmacokinetics
PS	Polystyrene
S	Solubility
V_{mean}	Mean ensemble velocity

REFERENCES

- Loira-Pastoriza C, Todoroff J, Vanbever R. Delivery strategies for sustained drug release in the lungs. *Adv Drug Deliv Rev.* 2014; 75:81–91. [PubMed: 24915637]
- Olsson, B.; Bondesson, E.; Borgström, L.; Edsbäcker, S.; Eirefelt, S.; Ekelund, K., et al. Pulmonary drug metabolism, clearance, and absorption.. In: Smyth, HDC.; Hickey, AJ., editors. *Controlled pulmonary drug delivery.* Springer; New York: 2011. p. 21-50.
- Jones RM, Neef N. Interpretation and prediction of inhaled drug particle accumulation in the lung and its associated toxicity. *Xenobiotica.* 2012; 42(1):86–93. [PubMed: 22106935]
- Wolff RK, Griffith WC Jr, Cuddihy RG, Snipes MB, Henderson RF, Mauderly JL, et al. Modeling accumulations of particles in lung during chronic inhalation exposures that lead to impaired clearance. *Health Phys.* 1989; 57(Suppl 1):61–7. Copyright (C) 2015 U.S. National Library of Medicine. discussion 7–8. [PubMed: 2481656]
- Gehr P, Blank F, Rothen-Rutishauser BM. Fate of inhaled particles after interaction with the lung surface. *Paediatr Respir Rev.* 2006; 7(Suppl 1):S73–5. [PubMed: 16798602]
- Schmid O, Moller W, Semmler-Behnke M, Ferron GA, Karg E, Lipka J, et al. Dosimetry and toxicology of inhaled ultrafine particles. *Biomarkers.* 2009; 14(Suppl 1):67–73. [PubMed: 19604063]
- Ibrahim M, Garcia-Contreras L. Mechanisms of absorption and elimination of drugs administered by inhalation. *Ther Deliv.* 2013; 4(8):1027–45. [PubMed: 23919477]
- Lai SK, Wang YY, Hanes J. Mucus-penetrating nanoparticles for drug and gene delivery to mucosal tissues. *Adv Drug Deliv Rev.* 2009; 61(2):158–71. [PubMed: 19133304]
- Nance EA, Woodworth GF, Sailor KA, Shih T-Y, Xu Q, Swaminathan G, et al. A dense poly(ethylene glycol) coating improves penetration of large polymeric nanoparticles within brain tissue. *Sci Transl Med.* 2012; 4:149ra119.
- Suk JS, Kim AJ, Trehan K, Schneider CS, Cebotaru L, Woodward OM, et al. Lung gene therapy with highly compacted DNA nanoparticles that overcome the mucus barrier. *J Control Release.* 2014; 178:8–17. [PubMed: 24440664]
- Yang M, Yu T, Wang YY, Lai SK, Zeng Q, Miao B, et al. Vaginal Delivery of paclitaxel via nanoparticles with non-mucoadhesive surfaces suppresses cervical tumor growth. *Adv Healthc Mater.* 2013
- Schopf LR, Popov AM, Enlow EM, Bourassa JL, Ong WZ, Nowak P, et al. Topical ocular drug delivery to the back of the eye by mucus-penetrating particles. *Transl Vis Sci Technol.* 2015; 4(3): 11. [PubMed: 26101724]
- Schopf L, Enlow E, Popov A, Bourassa J, Chen H. Ocular pharmacokinetics of a novel loteprednol etabonate 0.4% ophthalmic formulation. *Ophthalmol Ther.* 2014; 3(1–2):63–72.
- Baudy RB, Butera JA, Abou-Gharbia MA, Chen H, Harrison B, Jain U, et al. Prodrugs of perzinfotel with improved oral bioavailability. *J Med Chem.* 2009; 52(3):771–8. [PubMed: 19146418]
- Maury J, Feray L, Perfetti P, Bertrand MP. A flexible, stereoselective dimethylzinc-mediated radical-anionic cascade: dramatic influence of additional Lewis acids. *Org Lett.* 2010; 12(16): 3590–3. [PubMed: 20666447]
- Popov A, Enlow EM, Bourassa J, Gardner CR, Chen H, Ensign LM, et al. Nanocrystals, compositions, and methods that aid particle transport in mucus. 2013 US Patent Application Publication. US20130323179.
- Wang YY, Lai SK, Suk JS, Pace A, Cone R, Hanes J. Addressing the PEG mucoadhesivity paradox to engineer nanoparticles that “slip” through the human mucus barrier. *Angew Chem Int Ed Engl.* 2008; 47(50):9726–9. [PubMed: 18979480]
- Viaene E, Chanteux H, Servais H, Mingeot-Leclercq MP, Tulkens PM. Comparative stability studies of antipseudomonal beta-lactams for potential administration through portable elastomeric pumps (home therapy for cystic fibrosis patients) and motor-operated syringes (intensive care units). *Antimicrob Agents Chemother.* 2002; 46(8):2327–32. [PubMed: 12121900]

19. Lai SK, O'Hanlon DE, Harrold S, Man ST, Wang YY, Cone R, et al. Rapid transport of large polymeric nanoparticles in fresh undiluted human mucus. *Proc Natl Acad Sci U S A*. 2007; 104(5):1482–7. [PubMed: 17244708]
20. Tang BC, Dawson M, Lai SK, Wang YY, Suk JS, Yang M, et al. Biodegradable polymer nanoparticles that rapidly penetrate the human mucus barrier. *Proc Natl Acad Sci U S A*. 2009; 106(46):19268–73. [PubMed: 19901335]
21. Boer F. Drug handling by the lungs. *Br J Anaesth*. 2003; 91(1):50–60. [PubMed: 12821565]
22. Fukasawa M, Sumita Y, Harabe ET, Tanio T, Nouda H, Kohzuki T, et al. Stability of meropenem and effect of 1 beta-methyl substitution on its stability in the presence of renal dehydropeptidase I. *Antimicrob Agents Chemother*. 1992; 36(7):1577–9. [PubMed: 1510457]
23. Hirota T, Nishikawa Y, Tanaka M, Igarashi T, Kitagawa H. Characterization of dehydropeptidase I in the rat lung. *Eur J Biochem*. 1986; 160(3):521–5. [PubMed: 3780719]
24. Cayston prescribing information. Available from: http://www.cayston.com/prescribing_info.html as of August, 2014
25. Patel PR, Cook SE. Stability of meropenem in intravenous solutions. *Am J Health Syst Pharm*. 1997; 54(4):412–21. [PubMed: 9043564]
26. Teitelbaum AM, Meissner A, Harding RA, Wong CA, Aldrich CC, Remmel RP. Synthesis, pH-dependent, and plasma stability of meropenem prodrugs for potential use against drug-resistant tuberculosis. *Bioorg Med Chem*. 2013; 21(17):5605–17. [PubMed: 23845282]
27. Langer R. Polymer-controlled drug delivery systems. *Acc Chem Res*. 1993; 26(10):537–42.
28. Cystic Fibrosis Foundation Patient Registry 2008 Annual Data Report. Cystic Fibrosis Foundation; Bethesda: 2009.
29. Dales L, Ferris W, Vandemheen K, Aaron SD. Combination antibiotic susceptibility of biofilm-grown *Burkholderia cepacia* and *Pseudomonas aeruginosa* isolated from patients with pulmonary exacerbations of cystic fibrosis. *Eur J Clin Microbiol Infect Dis*. 2009; 28(10):1275–9. [PubMed: 19575248]
30. Maiz L, Giron RM, Oliveira C, Quintana E, Lamas A, Pastor D, et al. Inhaled antibiotics for the treatment of chronic bronchopulmonary *Pseudomonas aeruginosa* infection in cystic fibrosis: systematic review of randomised controlled trials. *Expert Opin Pharmacother*. 2013; 14(9):1135–49. [PubMed: 23586963]
31. Turnidge JD. The pharmacodynamics of beta-lactams. *Clin Infect Dis*. 1998; 27(1):10–22. [PubMed: 9675443]
32. Oermann CM, Retsch-Bogart GZ, Quittner AL, Gibson RL, McCoy KS, Montgomery AB, et al. An 18-month study of the safety and efficacy of repeated courses of inhaled aztreonam lysine in cystic fibrosis. *Pediatr Pulmonol*. 2010; 45(11):1121–34. [PubMed: 20672296]
33. Sahm D. In vitro activity of doripenem. *Clin Infect Dis*. 2009; 49(Suppl 1):S11–6. [PubMed: 19619017]
34. Gibson RL, Retsch-Bogart GZ, Oermann C, Milla C, Pilewski J, Daines C, et al. Microbiology, safety, and pharmacokinetics of aztreonam lysinate for inhalation in patients with cystic fibrosis. *Pediatr Pulmonol*. 2006; 41(7):656–65. [PubMed: 16703579]

Scheme 1.
Synthesis of MRPD starting from meropenem.

Fig. 1.

Wet milling of **MRPD** crystals in poloxamer 407 reduced the particle size from 100 nm to sub-micron by dynamic light scattering (DLS). Images were obtained using polarized light microscopy and scanning electron microscopy, respectively. Measurement by DLS shows that **MRPD-MPC** nanoparticles have average size (by intensity) of 443 nm, polydispersity index=0.173.

Fig. 2.

Time-course metabolism data shows that **MRPD** hydrolyzed into meropenem upon incubation in human lung microsomes at 37°C. The hydrolysis half-life was less than 0.5 h and reached 98% completion after 1.5 h.

Fig. 3.

The pharmacokinetics of meropenem ($n=3$ per time point) exhibit markedly different profiles in the lung depending on the formulation. When dosed as a prodrug suspension, the meropenem levels remained steady, ranging 1,072 to 2,727 ng/mL over 12 h after dosing. The prodrug levels were also sustained at 5,344 ng/mL during the entire observation period. In contrast, meropenem was rapidly eliminated when dosed as solution, reaching only 12 ng/mL after 9 h post-dose.

Fig. 4.

Proposed mechanism of drug transport in the airway after intratracheal instillation. **(a) MRPD-MPC:** after instillation, nanoparticles that avoided mucociliary clearance penetrate the fast-clearing luminal mucus gel (**a-I**) and deposit into the underlying periciliary layer (PCL) (**a-II**). Following slow nanoparticle dissolution (**a-III**), solubilized MRPD either absorb as intact prodrug or metabolize into meropenem (**a-IV**). The formation of a MRPD-MPC depot in the PCL (**a-II**) and slow nanoparticle dissolution (**a-III**) results in the sustained production of meropenem. **(b) Solution of meropenem:** after instillation, solubilized drug enters the airway and spreads in the luminal mucus layer (LML) (**b-I**), which subsequently either passively diffuses into the PCL (**b-II**) or eliminates via mucociliary clearance. Drugs that reach the PCL are then eliminated by metabolism and absorption into systemic circulation, which, altogether, results in short local drug duration.

Table 1Mobility of **MRPD-MPC** and control nanoparticles in CVM

Particle ensemble	$V_{\text{mean}}, \mu\text{m/s}$	Displacement from origin, μm	n
MRPD-MPCs	3.2 ± 1.1	2.7 ± 1.8	82
200-nm PS conjugated with mPEG-5000 (positive control)	6.6 ± 0.6	6.0 ± 2.8	45
200-nm carboxylate-terminated PS (negative control)	0.58 ± 0.80	0.29 ± 0.85	127

The velocities of the ensemble (V_{mean}) were determined by analysis of particle trajectories that were obtained using fluorescent microscopy. The particle population (n) per ensemble ranged from 45 to 127.