Research Article

Slow Release Formulations of Inhaled Rifampin

Intira Coowanitwong,¹ Vikram Arya,¹ Poj Kulvanich,² and Günther Hochhaus^{1,3}

Received 24 July 2007; accepted 27 May 2008; published online 27 June 2008

Abstract. Rifampin microspheres were prepared by spray drying using either polylactic acid (PLA) or poly(lactic-co-glycolic acid) (PLGA) polymers in different drug to polymer ratios (90:10 to 5:95, w/w). The *in-vitro* release characteristics, particle-size distribution, and cytotoxicity (in an alveolar macrophage cell line) and pharmacokinetics in rats after pulmonary instillation were evaluated. Increasing the polymer content from 10% to 95% slowed down the *in vitro* drug release with PLGA particles showing a steeper change with increasing polymer content (100% to 20% drug release over 6 h) than PLA particles (88% to 42% drug release over 6 h). PLA microsphere formulations revealed lack of cytotoxicity and a mass median aerodynamic diameter (MMDA) of 2.22–2.86 μm , while PLGA particles were larger (MMDA of 4.67–5.11 μm). Pharmacokinetics differed among the formulations with the 10% PLA formulation showing a distinct sustained release (t_{max} of 2 h vs 0.5 h of free drug) and a systemic bioavailability similar to that of free drug. Formulations with high polymer content showed a lower relative bioavailability (30%). This suggested that an optimal release rate existed for which a distinct amount of drug was delivered over an extended period of time.

KEY WORDS: cytotoxicity; inhalation; microspheres; pharmacokinetics; rifampin; sustained release.

INTRODUCTION

Tuberculosis (TB) remains a major cause of mortality. Approximately one of three of people worldwide are believed to be infected with the bacteria *Mycobacterium tuberculosis* (MTB) (1). Both the high prevalence of TB in patients infected with the human immunodeficiency virus (HIV) and the alarming increase in drug resistance have made tuberculosis (TB) an emerging threat. In 1993 the World Health Organization (WHO) declared TB a global public health emergency based on the increasing number of infected patients and the high mortality rate (2,3). According to WHO, the largest number of new TB cases in 2004 occurred in the Southeast Asia region, which accounted for 33% of incident cases around the world (4).

A variety of drugs have been used in combination for the treatment of MTB in order to ensure successful treatment and prevent the development of resistance (5,6). The drugs that comprise the first-line of defense include isoniazid, rifampin, pyrazinamide, streptomycin, and ethambutol. Isoniazid, rifampin, pyrazinamide, and streptomycin have bactericidal action while the ethambutol is bacteriostatic (7). Isoniazid and streptomycin are pharmacologically active against MTB only in the extracellular environment, where

the bacilli grow most rapidly along the cavity wall. Pyrazinamide is most effective in the acidic pH (pH 5.5) of the intracellular environment of the macrophages. Rifampin is the only anti-TB drug which, in addition to the activity *via* the aforementioned mechanisms, shows bactericidal activity in the extracellular environment where the slower growing semi-dormant organisms may demonstrate intermittent activity (8,9). Because of its favorable pharmacokinetics and unique mechanism of action, rifampin appears to be a suitable candidate for TB therapy, however it has been shown that chronic oral and i.v. therapy of rifampin resulted in adverse systemic side effects (such as rash, fever, and gastrointestinal disturbances, various symptoms related to the nervous system, and development of jaundice (7)) leading to poor patient compliance (7). Hence, alternate therapies are required that can reduce these concomitant side effects and help to localize the drug at the site of infection i.e., the lung.

The sustained release dosage form was selected over the uncoated, immediate release form for the following reasons: (a) Increase in the local effects due to application at the site of infection. (b) Requirement of relatively small doses for effective therapy. (c) Reduction in systemic exposure (due to local application) leading to a reduction of systemic adverse effects (10,11). All these factors are expected to result in a higher benefit-to-risk ratio and better patient compliance.

In this study, we investigated the use of a sustained release form of inhaled rifampin for TB therapy using animal models. Although there are several techniques to prepare the sustained release formulations, we selected a spray-drying method since it is a rapid, one-step process that converts liquid droplets to dried particulate form that can be efficiently inhaled (12–19). Thus, this approach can achieve a sustained

¹ Department of Pharmaceutics, College of Pharmacy, University of Florida, Gainesville, Florida 32610, USA.

² Department of Industrial Pharmacy, College of Pharmacy, Chulalongkorn University, Bangkok, Thailand.

³ To whom correspondence should be addressed. (e-mail: hochhaus@cop.ufl.edu)

presence of rifampin in the lung (increased pulmonary residence time) with sufficient pharmacological drug concentrations. The pulmonary fate of inhalation drugs is rather complex and it is affected by a number of pharmacokinetic/biopharmaceutical factors. In the case of slow-release formulations, it will depend, among other factors, on the release rate of the pharmacologically active drug and the pulmonary clearance of the particles, mainly due to the mucociliary clearance and other factors such as deposition efficiency and location. Here, we report the design of slow release, biodegradable rifampin particles that could be inhaled and our investigation of the relationship between the *in vitro* release rate and the pulmonary/systemic exposure.

MATERIALS

Rifampin was purchased from PCCA Inc. (Houston, TX, USA).

Poly(lactic acid) (PLA) or poly(lactic-co-glycolic acid) (PLGA) MW=75,000–125,000 and 40,000–65,000 Da, respectively) were acquired from Birmingham Polymers, Inc. (Birmingham, AL, USA). Dichloromethane was purchased from Shell Chemical (Bangkok, Thailand). Acetonitrile and methanol (HPLC grade), sodium chloride, dibasic sodium phosphate (anhydrous), monobasic sodium phosphate (anhydrous), and ascorbic acid were obtained from Fisher Scientific Inc. (Suwanee, GA, USA). Extra-fine lactose monohydrate (particle size of 10–30 μm) was donated from EM Industries (Hawthorne, NY, USA). Number 2 capsules were provided by Pfizer Inc. (Greenwood, SC, USA). J774A.1 murine macrophage (MTB strain H37Ra), Dulbecco's Modified Eagle's Medium (DMEM), phosphate buffer saline solution (PBS), and fetal bovine serum (FBS) were obtained from the American Type Culture Cell Collection (ATCC, Rockville, MD, USA). Middlebrook 7H9 and Middlebrook ADC Enrichment were purchased from BD Diagnostic Systems (Sparks, MD). Sodium chloride, Tween 80, microscope slides, and a Bacto Fluorescent Stain Set M were acquired from Fisher Scientific Inc. (Suwanee, GA, USA). 3-(4, 5-dimethylthiazole-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) was purchased from Sigma Chemical Co. (St. Louis, MO, USA). Male Sprague Dawley rats were obtained from Harlan Inc. (Indianapolis, IN, USA).

METHODS

Microsphere Formulations

Different ratios of PLA and PLGA polymers to rifampin (Table I) were dissolved in 4 L of methylene chloride. The solutions were fed through a high-speed rotating spray nozzle of a Mobile Minor spray dryer (Niro, Switzerland) to produce small droplets (10–15 μm). The flow rate of the solution was controlled at 15 mL/min and the rotating pressure of the nozzle was at 4 bars. The temperature in the chamber (50°C) was higher than the boiling point of methylene chloride (47°C) enabling microsphere dry powders to be obtained in the collector after the methylene chloride evaporated. In addition, drug free PLA and PLGA microspheres, as well as polymer-free rifampin microspheres were prepared accord-

Table I. Percent Undissolved Drug Remaining in Sustained-release Rifampin Formulations After 6 h

Polymer	Percent undissolved drug
Microspheres (PLA)	
PLA10	12.3 $\pm$ 3.2
PLA20	18.6 $\pm$ 2.6
PLA30	19.6 $\pm$ 3.4
PLA50	19.7 $\pm$ 1.8
PLA90	30.6 $\pm$ 1.1
PLA95	57.7 $\pm$ 0.5
Microspheres (PLGA)	
PLGA10	0
PLGA20	0
PLGA30	0
PLGA50	50.1 $\pm$ 6.3
PLGA90	76.7 $\pm$ 3.0
PLGA95	79.8 $\pm$ 1.6

ingly, using methylene chloride solutions of either polymer or drug.

Product Characterization

The information on particle size, shape, and surface morphology of the particles was obtained by S-4000 Fe-SEM Hitachi scanning electron microscope (Hitachi, USA). The samples were coated with gold using ion sputtering for 45 min prior to the experiment. Characterization was performed at 1–2 kV in a vacuum using different magnifications ($\times 35$, 50, and 100). An Image Pro Optical Analysis (MediaCybernetics Inc., USA) at two magnifications ($\times 35$ and $\times 100$) was employed. Densities (ρ) of particles at 25°C were determined with an Ultrapycometer 100 (Quantochrome, Boynton Beach, FL, USA). Helium (purity 99.9%) was used as the test gas.

Rifampin Analysis during Particle Characterization

The drug concentration in samples derived from pharmacokinetic, drug content and drug release samples was determined by reverse-phase HPLC (RP-HPLC) consisting of a Perkin Elmer Series 3B pump (Perkin Elmer Inc., USA) with a flow rate of 1.0 mL/min and a Waters C-18 (15 cm $\times$ ID 4.6 mm) column (Waters Co., USA) connected to a Perkin Elmer ISS100 autoinjector (Perkin Elmer Inc., USA). The detector was a Milton Roy SM-4000 (Milton Roy Co., USA) attached to a Hewlett Packard HP-3394A integrator (Hewlett-Packard Co., USA). The eluent was monitored at $\lambda=254$ nm. The mobile phase consisted of 50% acetonitrile in water with an additional 0.03% (v/v) trifluoroacetic acid (TFA). Six standard concentrations of rifampin (1, 2.5, 5, 10, 25, and 50 $\mu\text{g/mL}$) were prepared in the phosphate buffer pH 7.4, with 0.02% (v/v) of ascorbic acid as an antioxidant. An aliquot of 100 μL of methylprednisolone acetate (MPA) (100 $\mu\text{g/mL}$) was added into each 1 mL of the standard solutions, resulting in a final concentration of 10 $\mu\text{g/mL}$. The calibration curve was obtained using peak heights of the standard solutions.

Release Characteristics

In vitro dissolution of free drug and dry powder formulations (500 mg of 2% rifampin drug in lactose) was tested using the USP dissolution instrument (Vankel Technology Group, USA). One liter of phosphate buffer solution (pH 7.4) with 0.02% ascorbic acid as antioxidant was used as a dissolution medium. The dissolution apparatus was operated at 37°C using a rotating speed of 40 rpm. One milliliter of the sample was withdrawn at various time points for 24 h (0, 2, 5, 10, 15, 30 min and 1, 1.5, 2, 3, 6, 10, 20 and 24 h) and assayed by HPLC. The same quantity of medium was added immediately after each sample withdrawal to keep the volume of the medium constant. A graph of percent-undissolved drug *versus* time was plotted.

Cytotoxicity in Healthy Lung Macrophages

The fully adherent murine alveolar monocyte-macrophage cells (J774A.1) were grown in a DMEM supplement with 10% FBS without antibiotics at 37°C and 5% CO₂. Cytotoxicity tests were performed using the [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] MTT assay (20). Macrophages were seeded in a 96 well plate (Becton-Deckinson Labware, USA) with a concentration of 1×10^5 cells per well and were allowed to recover for approximately 24 h. Different rifampin concentrations (1,000, 100, 10, 1, 0.1, and 0.01 µg/mL) of free and sustained-release formulations, suspended in culture media were added. After 24 h, 25 mL of MTT solution (prepared in the PBS with final concentration of 5 mg/mL) was added. After 3 h of incubation, the supernatant was removed and 200 mL of dimethyl sulfoxide (DMSO) was added to each well. Finally, the purple formazan crystals were dissolved using a plate shaker (Biometra, Germany) for 30 min. The absorbance was measured using a microplate spectrophotometer model Spectramex250 (Molecular Devices, Sunnyvale, CA, USA) at a wavelength of 570 nm.

Fine Particle Fraction

An eight-stage cascade impactor (Graseby Anderson, USA) was used to determine the fine particle fraction of the rifampin formulations. Eight metal plates were installed on the impactor machine before use. A throat piece (non-USP type) that simulates the human throat was connected on the top of the device. The dose (containing 70 mg of free drug) was weighed, loaded directly into the chamber using a respirable device, Cyclohaler™ (Novartis Pharma AG, Switzerland), and dispersed at an inspiration rate of 28.3 L/min (confirmed by a flow meter, Bios International, USA) for an inhalation time of 1 min. After each determination, the powder on each plate and throat piece of the impactor was dissolved with 10 mL methanol, containing 0.02% (w/v) ascorbic acid solution and analyzed by reverse phase HPLC. The experiment was performed in triplicate. The percentage of the total dose collected from stages 2 to 5, representing particles with the aerodynamic diameter 1.1–5.8 µm, was considered to be the fine particle fraction (21). Graphs between the percent cumulative of rifampin were plotted against the effective cut off diameter for that stage (9.0, 5.8,

4.7, 3.3, 2.1, 1.1, 0.7 and 0.4 µm for stage 0 to 7, respectively). A 100% drug cumulative was calculated as the total amount of drug deposited on the entire cascade impactor. Calculation of the mass median aerodynamic diameter (MMAD) of the formulations was performed using R program (R Foundation, USA).

Animal Procedures

The project was approved by the Animal Care Committee, University of Florida, an AAALAC approved facility. Male Sprague Dawley rats (Harlan, Inc., Indianapolis, IN, USA), weighing 250–330 g, were housed for 12 h in a light/dark, constant temperature environment prior to the experiment. They were fed a standard pellet diet and water as required but were food-fasted overnight prior to each experiment.

Each rat was weighed and placed in a rat holder for intraperitoneal administration of anesthetics (fresh preparation of the combination of 1.5 mL of 10% (v/v) ketamine, 1.5 mL of 2% (v/v) xylazine, and 0.5 mL of 1% (v/v) acepromazine, at the dose of 1 mL/kg). The depth of the anesthesia was checked by tail pinch or pedal withdrawal reflex.

After the rats were completely anesthetized, one inch of a special round-tipped cannula attached to a delivery device for administration of the dry powders (Penn-Century Inc., Pennsylvania, USA) was introduced into the trachea through the mouth. A mixture of 10 ± 0.5 mg rifampin formulation in extra-fine lactose (to allow a similar insufflation volume for the different formulations), equivalent to 600 µg of rifampin free drug was placed in the chamber of the device and instilled into the lungs with insufflations of 3 mL of air. The rats were decapitated at 0.5, 1, 2, 4, 6, and 8 h after drug administration and 5 mL of blood was collected from each rat. The blood was centrifuged at 4°C for 15 min to obtain the plasma and 0.02% ascorbic acid (w/v) was added to the plasma. The plasma was snap frozen and stored at –70°C until further processing. Since rifampin has been shown to be very stable in plasma at –20°C (22,23), this procedure was judged to provide sufficient stability.

Plasma samples were thawed at room temperature and 25 µL of MPA (10 µg/mL) was added to 0.5 mL aliquots as an internal standard, resulting in a final concentration of 0.5 µg/mL. Ethanol (0.5 mL of 30%) was added to the plasma samples for protein precipitation. The samples were refrigerated for 15 min then centrifuged for 15 min at 4°C at $3,000 \times g$ using a CR422 centrifuge (Jouan Inc., USA). Endcapped Supelco C-8 cartridges (3 mL, Sigma-Aldrich Co., USA) were washed with 2 mL of water and 2 mL of 30% methanol and 1 mL of the supernatant was transferred onto the cartridges for solid phase extraction. The analytes were eluted from the column with 3 mL ethyl acetate. After 3 h evaporation, 100 µL of mobile phase was added to each tube for reconstitution. After centrifuging for 3 min at $6,000 \times g$ the drug concentration in the reconstituted samples was determined using the reverse phase high performance liquid chromatography (RP-HPLC) setup consisting of a Perkin Elmer Series 3B pump (Perkin-Elmer Inc., USA) with a flow rate of 1.0 mL/min and a C-18 (30 cm $\times$ 4.6 mm) column (Waters, USA) connected to a Perkin Elmer ISS200 autoinjector. The detector was an SPD-10A

(Shimadzu, USA) attached to a computer system. Turbochrom Navigator[®] software version 4.0 was used for the data analysis (Perkin-Elmer Inc., USA). The eluent was monitored at $\lambda = 254$ nm. The mobile phase consisted of a mixture of 35:65 acetonitrile to pH 3.5-phosphate buffer. The calibration curve for rifampin was linear in the range of 8 ng/mL to 0.5 $\mu\text{g/mL}$ ($r^2 > 0.995$), with linearity extending well beyond this range. Slopes from the calibration curves were used to calculate sample concentrations. This assay was validated to ensure both precision and accuracy in accordance with the FDA guidelines for bioanalytical method validation (24). The intra-day precision (percent coefficient of variation [CV]) and accuracy (percent bias) of the quality control (QC) samples were 8.49–11.9% and 101.9–102.4%, respectively.

Pharmacokinetic Analysis

The plasma concentration vs time data, obtained after intratracheal instillation of free rifampin and rifampin microspheres were analyzed using the noncompartmental model (extravascular input) in WinNonlin[®] (Pharsight Corp, USA). The various pharmacokinetic parameters estimated were: maximum plasma concentration (C_{max}), time to reach maximum concentration (T_{max}), area under the curve from time zero to last time point of the experiment (AUC_{0-t}). The relative bioavailability (F_{rel}) for each formulation was calculated as:

$$F_{\text{rel}} = \frac{\text{AUC}_{\text{SusRIF}}}{\text{AUC}_{\text{FreeRIF}}} \quad (1)$$

where $\text{AUC}_{\text{SusRIF}}$ is the area under the curve of the sustained-release formulations and the $\text{AUC}_{\text{FreeRIF}}$ is the area under the curve of the free drug.

Statistics

The difference in pharmacokinetic parameters and respirable fractions between each formulation was tested for significance using ANOVA (Design Expert version 6 Software for Experiment Design, Stat Ease Inc., USA) assuming $\alpha = 0.05$.

RESULT AND DISCUSSION

Morphology

Scanning electron microscopy (SEM) of the microspheres showed morphological differences directly related to the type and content of the polymers. Free drug microspheres showed a number of fractures thereby indicating the lack of particle elasticity and flexibility (data not shown). Figure 1 shows the rifampin microsphere formulations containing 0% to 100% of the PLA and PLGA, respectively. The microspheres of PLA and PLGA bulk polymer were spherical with rounder edges, but of shriveled and raisin-like shape (Fig. 1). Microspheres with increasing polymer content had a more defined round shape indicating that polymers can help to increase the elasticity and flexibility of the particles. It should be noted however, that the presence of shriveled and raisin-like shape microsphere might be due to the lack of a plasticizer. We did

not use a plasticizer in our experiments due to the possible toxic residue in the lung after inhalation of the formulations. Based on SEM measurements, the sizes of PLA and PLGA was comparable.

In Vitro Release Characteristics

The dissolution profiles of the microspheres prepared with various contents of PLA and PLGA are shown in Figs. 2 and 3.

Fig. 1. SEM micrograph of rifampin microspheres containing PLGA and PLA

Fig. 2. Release profiles of free drug compared to rifampin microspheres containing 10–95% PLA

The dissolution profiles of the microsphere formulated with high polymer content showed that the drug was not able to provide complete (100%) release. This may be due to the fact that the dissolution medium could not permeate through the polymer network and dissolve the drug molecules within the matrix system. Alternatively, the drug solution was trapped inside the polymer network and could not diffuse to the dissolution medium within 24 h. The results further indicated a biphasic release profile of the drug and showed the tendency of the release rate to follow zero order kinetics (25). The percent of the drug not being released from the microspheres after 24 h is shown in Table I. It is recognized that additional release might be observed in biological systems where the microsphere breakdown might be facilitated by enzymes.

The results indicated a relationship between the polymer content of the formulations and the release rate of the drug from the microspheres, with higher polymer loads providing slower release rates. Literature data (25) showed that increasing the polymer content of three polymers (Kollidon SR, hydroxypropyl methylcellulose, and carnuba wax) in formulations of theophylline, diclofenac sodium, and diltiazem HCL) prolonged the sustained release characteristics.

Fig. 3. Release profiles of free drug compared to rifampin microspheres containing 10–95% PLGA

This might be due to decreased porosity (pore or channel between the particles) and increased tortuosity (branching and bending of the pores). The type of polymer is also an important factor which influences the release characteristics of the formulations (25). Results show that polymers which exhibit the maximum hydrophobicity (carnuba wax) show the maximum sustained release characteristic. In our study, the profiles of microspheres containing PLA show a slower release rate compared to the microspheres coated with PLGA. This can be attributed to the higher crystallinity of the PLA as compared to PLGA. However, this hypothesis was only applicable for the formulations with low PLA contents (10–30%). The profiles with higher PLA content (50–95%) unexpectedly showed faster release rates compared to the microspheres containing PLGA. However, differences in the particle size and particle size distribution may also be a contributing factor.

Fine Particle Fraction

Figures 4 and 5 show the particle-size distribution pattern of the rifampin microspheres containing different percentages of PLA and PLGA, respectively. The majority of PLA coated particles were found on stages 2–5, representing the aerodynamic diameter in the range of 1.1–5.8 μm . However, the PLGA coated particles were deposited primarily on the throat and stage 0–1, representing an aerodynamic diameter in the range between 5.8 and 10 μm . Table II shows that the fine particle fraction of the free drug and spray-dried microsphere formulations containing both PLA and PLGA were significantly different ($P < 0.05$).

There were significant differences between the geometric diameters obtained by SEM and the aerodynamic diameter of the microsphere formulations. The SEM-based particle size of the microspheres prepared from PLA and PLGA were both in the range between 5 and 15 μm (Fig. 1) while the MMAD of the microspheres obtained with the cascade impactor were in the range of 2.22 to 2.86 μm for the PLA (significantly different to free drug, $P < 0.05$) and in the range of 4.65 to 5.11 μm for the PLGA particles (not significantly different to free drug, $P > 0.05$; Table II). The significantly lower fine particle fraction and higher MMAD of PLGA particles as

Fig. 4. Particle-size distribution of free drug compared to rifampin microspheres containing 10–95% PLA

Fig. 5. Particle-size distribution of free drug compared to rifampin microspheres containing 10–95% PLGA

compared to PLA particles, as determined by cascade impactor, was unexpected. SEM analysis did not reveal any differences in the morphology of the particles and there was no clearcut difference in the density of the different polymer formulations (Table II). Thus, the reason for this difference could not be identified at the present time.

Cytotoxicity in Healthy Lung Macrophages

Cell toxicity was investigated after applying 10 fold dilutions (0.01, 0.1, 1, 10, 100, to 1000 $\mu\text{g/mL}$) of the free drug and the coated rifampin prepared by PLA and PLGA.

The experiments showed no significant difference in percent cell viability of these high polymer content-formulations compared to the free drug at all drug concentrations. Although the experiments were not performed on formulations with lower polymer content (10–30%), it is reasoned that similar results would be obtained, since lower polymer content should have less of an effect on cells. From these studies, it appeared that, there was no additional toxicity of formulations composed of various polymer contents as compared to the free drug.

Table II. Percent Fine Particle Fraction, MMAD, and Densities of the Rifampin Formulations

Formulations	Fine particle fraction (%)	MMAD (μm)	Density (g/mL)
PLA Microspheres			
PLA10	66.9 $\pm$ 4.3	2.51 $\pm$ 0.14	0.84 $\pm$ 0.01
PLA20	66.3 $\pm$ 5.1	2.86 $\pm$ 0.50	0.80 $\pm$ 0.02
PLA30	55.2 $\pm$ 11.5	2.64 $\pm$ 0.43	0.82 $\pm$ 0.04
PLA50	66.8 $\pm$ 4.3	2.27 $\pm$ 0.09	0.83 $\pm$ 0.01
PLA90	68.4 $\pm$ 2.6	2.42 $\pm$ 0.37	0.72 $\pm$ 0.03
PLA95	67.4 $\pm$ 2.6	2.22 $\pm$ 0.37	0.62 $\pm$ 0.03
PLGA Microspheres			
PLGA10	30.5 $\pm$ 2.6	4.67 $\pm$ 0.23	1.00 $\pm$ 0.02
PLGA20	28.6 $\pm$ 0.8	4.91 $\pm$ 0.26	0.95 $\pm$ 0.02
PLGA30	31.0 $\pm$ 3.0	4.65 $\pm$ 0.21	1.00 $\pm$ 0.03
PLGA50	34.2 $\pm$ 2.1	4.65 $\pm$ 0.41	0.98 $\pm$ 0.01
PLGA90	26.5 $\pm$ 3.9	4.92 $\pm$ 0.34	0.70 $\pm$ 0.03
PLGA95	22.9 $\pm$ 3.9	5.11 $\pm$ 0.36	0.68 $\pm$ 0.06

Fig. 6. Plasma concentration vs time profiles of rifampin formulations in rats

Animal Experiments

Pharmacokinetic studies were included to assess potential differences in the pulmonary absorption, by evaluating area under the concentration time curves and C_{max} estimates. This was feasible as AUC estimates after pulmonary deposition reflect differences in the pulmonarily available drug (release from dosage form and absorption). Interpretation of the pharmacokinetic results was somewhat hampered by the small number of blood samples (five) that was collected, which was insufficient to describe absorption and elimination rigorously. Figure 6 shows the average plasma concentration as a function of time after intratracheal instillation of free and the selected sustained-release rifampin formulations. Pharmacokinetic parameters obtained after noncompartmental analysis of the plasma concentration vs time data are listed in Table III.

In agreement with a slower absorption, C_{max} values after all microsphere formulations were lower than after administration of pure rifampin with a tendency of higher polymer load resulting in lower C_{max} values. The AUC_{0-8} of the PLA10 was comparable to the results obtained for unencapsulated free drug ($P>0.05$) and significantly higher than of PLA30 and PLA95. This indicates that all the drug content of the PLA10 formulation was released and delivered to the lung in a sustained release fashion. The formulations with higher PLA content PLA 30 and PLA95, while showing more pronounced sustained delivery *in vitro*, produced a much lower degree of systemic absorption. The reduced systemic bioavailability is due to the fact that only a fraction of the encapsulated material is released from the formulation in the

Table III. Pharmacokinetic Parameters After Intratracheal Instillation of Various Rifampin Formulations

	Free RIF	PLA10	PLA30	PLA95
C_{max} ($\mu\text{g/mL}$)	6.97 $\pm$ 2.69	1.30 $\pm$ 0.02	0.66 $\pm$ 0.32	0.67 $\pm$ 0.02
T_{max} (h)	0.5	2.0	1.0	1.0
AUC_{0-8} ($\mu\text{g/mL h}$)	4.4 $\pm$ 1.5	5.7 $\pm$ 0.2	1.4 $\pm$ 0.3	1.3 $\pm$ 0.3
F_{rel}	1	1.2	0.3	0.3

lung, while the mucociliary clearance removes unreleased, trapped microencapsulated drug from the lung. Alternatively, drug particles might also be phagocytosed by the alveolar macrophages and removed (26). Therefore, slow release formulations for pulmonary delivery have to consider the potential removal from the lung by these clearance mechanisms. A reduced systemic availability has also been observed for slow dissolving lipophilic glucocorticoids that are removed by mucociliary clearance, especially when drug is deposited in more central areas of the lung (27).

Slow release formulations for oral rifampin have been described by other research groups. Ain *et al.* (28), using oral sustained-release rifampin formulation prepared using PLGA as the coating polymer, showed that the oral bioavailability of the sustained release drug was not altered while an increase in the terminal half-life indicated a sustained release behavior, suggesting flip-flop kinetics. Our results indicate that a sustained drug exposure can also be obtained with pulmonary formulations. In addition, the inhalation approach might allow a significantly higher pulmonary drug concentration.

CONCLUSIONS

By increasing the polymer content, both PLA and PLGA-containing microspheres were shown to be rounder and less broken than spray-dried rifampin. PLA and PLGA particles differed in the drug release rate, fine particle fraction, and MMAD compared to the free drug ($P < 0.05$). Because of the higher hydrophobicity of PLA, microspheres at low PLA content (10–30%) provided slower sustained release compared to PLGA microspheres with the same level of polymer content. However, at the high polymer content (50–95%), the PLA microspheres showed a faster release compared to the PLGA containing microspheres since the MMAD of PLA-containing microspheres was smaller. No overt toxicity of the microspheres was detected against macrophages. When compared to microsphere formulations with higher polymer content (30% and 95% PLA), the formulation with low polymer content (10% PLA) showed higher C_{max} , significantly higher AUC_{0-8} , and F_{rel} . These studies showed that local sustained delivery of rifampin is possible using slow release microspheres.

REFERENCES

1. Tuberculosis Worldwide. Health Protection Agency, 2006; http://www.hpa.org.uk/infections/topics_AZ/tb/worldwide.htm.
2. E. L. Barrow, G. A. Winchester, J. K. Staas, D. C. Quenelle, and W. W. Barrow. Use of microsphere technology for targeted delivery of rifampin to *Mycobacterium tuberculosis*-infected macrophages. *Antimicrob Agents Chemother.* **42**:2682–2689 (1998).
3. S. Connert. *TB sourcebook. The complete tuberculosis resource*, American Health Consultants, Georgia, 1997.
4. WHO. World Health Organization Factsheet N104, March 2006. <http://www.who.int/mediacentre/factsheets/fs104/en/print.html>.
5. J. D. Grodon. Antituberculous therapy. In L. I. Lutwick (ed.), *Tuberculosis. A clinical handbook*, Chapman & Hall, New York, 1995, pp. 295–316.
6. E. Mutschler, and H. Derendorf. *Drug Actions. Basic principles and therapeutic aspects*, CRC, Ann Arbor, MI, 1995.
7. P. E. Patterson, M. E. Kimerling, W. C. Bailey, and N. E. Dunlap. Chemotherapy of tuberculosis. In D. Schlossberg (ed.), *Tuberculosis and nontuberculous mycobacterial infections*, W.B. Saunders Co, PA, 1999, pp. 71–82.
8. L. N. Friedman. Pulmonary tuberculosis: presentation, diagnosis and treatment. In L. N. Friedman (Ed), *Tuberculosis: Current concepts and treatment*. CRC, New York, 2001, pp 107–138.
9. D. A. Mitchison. Basic mechanisms of chemotherapy. *Chest.* **76**(6 Suppl):771–781 (1979).
10. P. R. Byron. Prediction of drug residence times in regions of the human respiratory tract following aerosol inhalation. *J Pharm Sci.* **75**:433–438 (1986).
11. S. Suarez, R. J. Gonzalez-Rothi, H. Schreier, and G. Hochhaus. Effect of dose and release rate on pulmonary targeting of liposomal triamcinolone acetone phosphate. *Pharm Res.* **15**:461–465 (1998).
12. D. A. Edwards, J. Hanes, G. Caponetti, J. Hrkach, A. Ben-Jebria, M. L. Eskew, J. Mintzes, D. Deaver, N. Lota, and R. Langer. Large porous particles for pulmonary drug delivery. *Science.* **276**:1868–1871 (1997).
13. R. Vanbever, J. D. Mintzes, J. Wang, J. Nice, D. Chen, R. Batycky, R. Langer, and D. A. Edwards. Formulation and physical characterization of large porous particles for inhalation. *Pharm Res.* **16**:1735–1742 (1999).
14. R. Bodmeier, and H. G. Chen. Preparation of biodegradable poly(+/–)lactide microparticles using a spray-drying technique. *J Pharm Pharmacol.* **40**:754–757 (1988).
15. Y. Kawashima, S. Y. Lin, M. Ueda, H. Takenaka, and Y. Ando. Direct preparation of solid particulates of aminopyrine–barbital complex (pyrabital) from droplets by a spray-drying technique. *J Pharm Sci.* **72**:514–518 (1983).
16. Y. F. Maa, P. A. Nguyen, T. Sweeney, S. J. Shire, and C. C. Hsu. Protein inhalation powders: spray drying vs spray freeze drying. *Pharm Res.* **16**:249–254 (1999).
17. P. O'Hara, and A. J. Hickey. Respirable PLGA microspheres containing rifampicin for the treatment of tuberculosis: manufacture and characterization. *Pharm Res.* **17**:955–961 (2000).
18. H. Takenaka, Y. Kawashima, and S. Y. Lin. Preparation of enteric-coated microcapsules for tableting by spray-drying technique and *in vitro* simulation of drug release from the tablet in GI tract. *J Pharm Sci.* **69**:1388–1392 (1980).
19. L. S. C. Wan, P. W. S. Heng, and C. G. H. Chia. Spray drying as a process for microencapsulation and the effect of different coating polymers. *Drug Dev Ind Pharm.* **18**:997–1011 (1992).
20. T. Mosmann. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J Immunol Methods.* **65**:55–63 (1983).
21. A. J. Hickey, and T. B. Martonen. Behavior of hygroscopic pharmaceutical aerosols and the influence of hydrophobic additives. *Pharm Res.* **10**:1–7 (1993).
22. J. B. Lecaillon, N. Febvre, J. P. Metayer, and C. Souppart. Quantitative assay of rifampicin and three of its metabolites in human plasma, urine and saliva by high-performance liquid chromatography. *J Chromatogr.* **145**:319–324 (1978).
23. J. Woo, C. L. Wong, R. Teoh, and K. Chan. Liquid chromatographic assay for the simultaneous determination of pyrazinamide and rifampicin in serum samples from patients with tuberculous meningitis. *J Chromatogr.* **420**:73–80 (1987).
24. Food and Drug Administration. *Bioanalytical method validation. Guidance for industry*. US Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER), Center for Veterinary Medicine (CVM). <http://www.fda.gov/CDER/GUIDANCE/4252fnl.pdf>, 2001.
25. M. S. Reza, M. A. Quadir, and S. S. Haider. Comparative evaluation of plastic, hydrophobic and hydrophilic polymers as matrices for controlled-release drug delivery. *J Pharm Pharm Sci.* **6**:282–291 (2003).
26. R. Sharma, D. Saxena, A. K. Dwivedi, and A. Misra. Inhalable microparticles containing drug combinations to target alveolar macrophages for treatment of pulmonary tuberculosis. *Pharm Res.* **18**:1405–1410 (2001).
27. T. W. Harrison, and A. E. Tattersfield. Plasma concentrations of fluticasone propionate and budesonide following inhalation from dry powder inhalers by healthy and asthmatic subjects. *Thorax.* **58**:258–260 (2003).
28. Q. Ain, S. Sharma, S. K. Garg, and G. K. Khuller. Role of poly [DL-lactide-co-glycolide] in development of a sustained oral delivery system for antitubercular drug(s). *Int J Pharm.* **239**(1–2):37–46 (2002).