A Novel Inhalable Form of Rifapentine

JOHN G. Y. CHAN,[1] COLIN C. DUKE,[2] HUI XIN ONG,[1] JOSEPH C. Y. CHAN,[3] ANNELIESE S. TYNE,[4] HAK-KIM CHAN,[2] WARWICK J. BRITTON,[4,5] PAUL M. YOUNG,[1] DANIELA TRAINI[1]

[1]Respiratory Technology, Woolcock Institute of Medical Research and Discipline of Pharmacology, Sydney Medical School, The University of Sydney, Sydney, Australia
[2]The Faculty of Pharmacy, The University of Sydney, Sydney, Australia
[3]Sydney Medical School, The University of Sydney, Sydney, Australia
[4]Tuberculosis Research Program, Centenary Institute, Sydney, Australia
[5]Discipline of Medicine, Central Clinical School, Sydney Medical School, The University of Sydney, Sydney, Australia

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ABSTRACT: Recent murine studies found that rifapentine, dosed daily, at least halved tuberculosis treatment times compared with standard rifampicin and isoniazid-containing regimens. However, in humans, an inhalable form of rifapentine may be necessary to considerably shorten treatment duration because of the physiological barriers associated with oral therapy. The current study compares two inhalable rifapentine dry powders—a novel pure crystalline form and an amorphous form—by a series of *in vitro* tests. The crystalline and amorphous powders had a mass median aerodynamic size of 1.68 ± 0.03 and 1.92 ± 0.01 μm, respectively, associated with a fine particle fraction of 83.2 ± 1.2% and 68.8 ± 2.1%, respectively. A quinone degradation product was identified in the amorphous powder stored for 1 month, whereas the crystalline form remained chemically stable after storage at both 0% and 60% relative humidity, 25°C, for at least 3 months. Solubilized rifapentine was well tolerated by pulmonary tissue and macrophage cells up to approximately 50 μM. The accumulation of rifapentine within alveolar macrophage cells was significantly higher than for rifampicin, indicating enhanced delivery to infected macrophages. The novel inhalable crystalline form of rifapentine is suitable for targeted treatment of tuberculosis infection and may radically shorten treatment duration. © 2014 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 103:1411–1421, 2014

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INTRODUCTION

The introduction of shorter treatment regimens for tuberculosis could dramatically reduce disease incidence and associated mortality.[1] Standard treatment with a rifampicin-containing combination antibiotic regimen for active infection is currently 6 months, whereas the gold standard treatment for latent infection is 9 months of isoniazid.[2,3] Both treatment options are orally administered, daily regimens.

Rifapentine has several times longer serum half-life and greater bactericidal dosing compared with rifampicin, and is approved by the United States Food and Drug Administration as a first-line drug for once or twice-weekly dosing in the treatment of tuberculosis.[4] Importantly, recent studies in murine infection models found that much shorter treatment times for tuberculosis could be achieved when oral rifapentine was dosed daily and substituted for rifampicin in the treatment of active infection (3 months) and isoniazid in treating latent infection (2 months).[2,3] Thus, the substitution of current key antitubercular drugs (rifampicin and isoniazid) with rifapentine might radically alter tuberculosis treatment outcomes.

However, a subsequent human clinical trial[5] was unable to reproduce these shortened treatment times for active tuberculosis and was linked to insufficient concentrations in pulmonary granulomas. This was attributed to a variety of reasons including high rifapentine plasma protein binding (98%), variation in rifapentine bioavailability with food intake, and induction of its own metabolism.[4–6] Particularly concerning was the finding by Dooley et al.[4] in a multidose human rifapentine pharmacokinetic study that the exposure and maximum concentration of orally administered rifapentine was unchanged between the upper oral doses of 15 and 20 mg/kg. If exposure levels beyond those found at 15 mg/kg are necessary for treatment shortening, shorter therapies in human patients may be unachievable with current oral dosing.

These higher exposure levels might instead be achieved by other means such as direct aerosol delivery of rifapentine to the pulmonary site of infection. A rapidly growing cache of work has investigated formulation and administration of inhalable antitubercular drugs.[7–13] In mice, antitubercular drugs administered at a lower dose and dosing frequency as an aerosol, achieved similar efficacy to oral administration.[14] Further, a recent review thoroughly assesses the steps necessary for existing research to progress into clinical trials.[15] However, there are no previous studies investigating inhalable rifapentine, perhaps because evidence suggesting the considerable treatment shortening potential of rifapentine compared with other more commonly studied antitubercular drugs is still relatively recent.

Furthermore, a disadvantage of rifamycin-containing formulations is their tendency to undergo air oxidation to give a quinone degradation product.[16,17] It was previously shown that

Correspondence to: Assoc Prof Daniela Traini (Telephone: +61-2-91140352; Fax: +61-2-93514391; E-mail: daniela.traini@sydney.edu.au)

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a crystalline dihydrate form of rifampicin did not experience the rapid oxidation seen for its amorphous counterpart.[11] However, because of the differences in chemical structure between the two rifamycins, a crystalline dihydrate form of rifapentine could not be similarly generated by this method (unpublished data). In addition, published crystalline forms of rifapentine are limited to its methanol solvate, which are unsuitable for pharmaceutical use.[18,19] Hence, if efficacious pulmonary levels of rifapentine are to be achieved, there is a need to identify a suitable synthesis method for a pure form of crystalline rifapentine that is readily tailored to an inhalable size.

With the growing interest toward the use of rifapentine, the current work describes a novel inhalable form of crystalline rifapentine prepared using a simple three-step process that is suitable for clinical use to deliver high pulmonary rifapentine concentrations. This was compared with amorphous rifapentine microparticles produced by spray drying. In this manuscript, powder characteristics, aerosol performance, *in vitro* biological properties, and storage stability are analyzed and compared.

MATERIALS AND METHODS

Manufacture of Inhalable Rifapentine Dry Powders

A crystalline rifapentine dry powder was prepared by dissolving rifapentine (4 mg/mL, maximum solubility) (Hangzhou ICH Imp & Exp Company Ltd., Hangzhou, China) in a cosolvent system of acetone and deionized water (1:1, v/v) (Thermo Fisher Scientific, Scoresby, Australia; Milli-Q, Sydney, Australia). The cosolvent system was chosen to achieve reasonable rifapentine concentration using a relatively nontoxic solvent. Acetone was removed by heating the solution to 67°C, giving a primarily aqueous suspension of crystalline rifapentine. The suspension was homogenized at 10,000 rpm for two minutes (Silverson L4RT High Shear Mixer, Silverson, Chesham, United Kingdom) to obtain a smaller and more uniform crystal size. This suspension was then spray-dried using a B-290 Mini spray-dryer operated in open loop, connected in series with a Buchi-296 dehumidifier (all from Buchi Laboratories, Flawil, Switzerland). Spray-drying was undertaken with the following settings: inlet temperature 60°C, atomizer 55 mm (approximately 650 L/h), aspirator 100% (40 m^3/h), feed rate 5% (2 mL/min), and nozzle cleaner activated.

The amorphous rifapentine dry powder was manufactured by solubilizing rifapentine at 4 mg/mL in methanol and spray dried. The spray drier conditions were as above but operated in closed loop (Buchi-295 Inert Loop; Buchi Laboratories) with nitrogen used as the drying gas.

Scanning Electron Microscopy

The dry powders were imaged using scanning electron microscopy (Zeiss Ultra Plus scanning electron microscope, Carl Zeiss, Oberkochen, Germany) at an acceleration voltage of 5 kV. Powders were dispersed onto carbon tape and sputter-coated with 15 nm of gold (Emitech K550X).

Particle Sizing

Laser diffraction (Malvern Mastersizer 2000) was used in conjunction with a Scirocco 2000 dry powder accessory (both from Malvern Instruments Ltd., Worcestershire, UK) to determine the volumetric median diameter (VMD) and span (difference between the 10th and 90th percentile particle diameters) of the rifapentine dry powders. A dispersive air pressure of 3.5 bar was used for both powders after assessing changes in median particle size over a range of pressures (0.5–4.0 bar). Refractive index was set to 1.6 for rifapentine. Measurements were performed in triplicate.

Aerosol Performance

Aerosol performance of the dry powders was assessed by dispersion of the powder through a multistage liquid impinger (MSLI) (Copley Scientific, Nottingham, UK). The rinsing solvent consisted of a methanol:aqueous ascorbic acid (1 mg/mL) (75:25, v/v) solution and was analysed according to a validated method.[20] Briefly, 20 mL of rinsing solvent was filled into each of the four MSLI stages and a 0.2 μm glass filter (Pall Corporation, Sydney, Australia) fitted into the fifth stage. An Aeroliser® dry power inhalation (DPI) device (Novartis,North Ryde, Australia; Promega, Madison, United States; Molecular Devices, Sunnyvale, United States) was coupled to the United States Pharmacopoeia (USP) throat of the MSLI using a mouthpiece adapter. A size 3 hydroxypropyl methylcellulose capsule (Capsugel, Greenwood, United States) containing 10 mg of dry powder was actuated at an airflow of 100 L/min—calibrated using an airflow meter and flow controller (TSI 3063 airflow meter; TSI Instruments Ltd., Buckinghamshire, UK; Flutus Air, Novi Systems Ltd., Dulverton, UK)—through the DPI device for 4 s. Prespecified amounts of rinsing solvent were used postactuation to meticulously wash the device, capsule, throat, and stages 1–5 of the MSLI. Samples were tested in triplicate.

Drug quantification was assayed by HPLC, with a system composing of a CBM-20A controller, LC-20AT pump, SPD-20A UV/VIS detector, SIL-20A HT autosampler, and LCSolution software (Shimadzu Corporation, Kyoto, Japan) using a literature method.[20] A mobile phase consisting of phosphate buffer (pH 3.0):acetonitrile:tetrahydrofuran (53:42:5, v/v) (orthophosphoric acid from Ajax Finechem Pty Ltd., Taren Point, Australia; potassium dihydrogen orthophosphate from Biolab Ltd., Clayton, Australia; acetonitrile and tetrahydrofuran from Honeywell, Morristown, New Jersey) was run at an isocratic flow rate of 1 mL/min. The stationary phase was a μBondapak™ C18 (3.9 × 300 mm^2) column (Waters, Milford, Massachusetts), detection wavelength set at 256 nm, and sample injection volume of 20 μL. A standard curve for rifapentine was derived using standard solutions with concentrations from 0.001 to 0.4 mg/mL and had an R^2 value of 1.00.

The total emitted dose was defined as the weight difference between the initial loaded drug weight, and the weight of drug retained within the Aeroliser® and capsule following aerosolization. This was used to calculate the fine particle fraction (FPF) (percent mass of aerosol particles with an aerodynamic diameter <5 μm) and mass median aerodynamic diameter (MMAD), which was derived from the log-probability plot of the MSLI data.

X-Ray Powder Diffractometry

X-ray powder diffractometry (XRPD) (D5000; Siemens, Karlsruhe, Germany) was used to analyze crystallinity of the dry powders from 5° to 40° 2θ, using Cu Kα radiation (30 mA, 40 kV) at a step rate of 0.04° 2θ per second. The sample powders were placed into the cavity of a XRPD glass slide for analysis.

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