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Research paper

A novel dry powder inhalable formulation incorporating three first-line anti-tubercular antibiotics

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ABSTRACT

Treatment for tuberculosis (TB) using the standard oral antibiotic regimen is effective but inefficient, requiring high drug dosing and lengthy treatment times. Three concurrent first-line antibiotics recommended by the World Health Organization (WHO) guidelines are pyrazinamide, rifampicin and isoniazid. Combining these antibiotics in a novel formulation for dry powder inhalation (DPI) may facilitate rapid and efficient resolution of local and systemic infection. However, spray-dried individually, these antibiotics were found to be physically unstable. A solution of the three antibiotics, at the WHO-recommended ratio, was spray-dried. The collected powder was assessed by a series of *in vitro* methods to investigate aerosol performance, particle physico-chemical characteristics and dissolution profile. Particles obtained were spherical with a surface composed primarily of rifampicin, as identified by TOF-SIMS. A mass median aerodynamic diameter of 3.5 ± 0.1 μm and fine particle fraction (<5 μm) of 45 ± 3% indicated excellent aerosol performance. The combination powder was differentiated by the presence of rifampicin dihydrate and the delta polymorph of pyrazinamide. Quantitative analysis indicated individual particles contained the three antibiotics at the expected proportions (400:150:75 w/w). This excipient-free triple antibiotic DPI formulation could be used as a significant enhanced treatment for TB.

1. Introduction

Tuberculosis (TB) remains a significant medical issue worldwide, despite implementation of a highly effective standard treatment regimen. The World Health Organization recommended regimen [1] consisting of oral co-administered isoniazid, rifampicin, pyrazinamide and often ethambutol is primarily threatened by poor patient adherence [2,3]. This is partly related to side effects of oral administration, which require high drug dosing and lengthy treatment times. These high doses are necessary for the drugs to reach poorly or non-vascularised sections of the body such as granulomas, tubercles and infected alveolar macrophages [4]. The long treatment times (up to 6 months) treat the slow-growing populations of bacteria but also decrease patient adherence [5]. Premature self-termination of treatment by patients in turn leads to drug resistant strains of TB which further complicates treatment efficacy.

Inhalable anti-tubercular formulations have been proposed as a potential solution to these issues [6–15]. Furthermore, since tuberculosis is primarily communicated via the pulmonary route, with 75–80% of cases remain localised in the lungs [5,15,16], aerosolised therapy appears the most logical route. Pulmonary therapeutics increase the chance of arresting TB infection before dissemination to other organs by maximising drug concentration at infected sites in the lungs, thereby reducing overall drug dosing and related side effects [11,17,18]. These local drug concentrations may even be high enough to overcome some drug resistance [15]. More specifically, inhaled antibiotic particles can target alveolar macrophages which, when infected with TB, are maintained in a state of 'alternative activation' whereby macrophage cytosol conditions are suitable for TB bacilli replication and survival [8,15]. However, phagocytosis of microparticles has been shown to revert these macrophages to a 'classical activation' state that resurrects their innate bacterial clearance mechanisms [6,14,15,19]. Thus, inhaled antibiotic microparticles are expected to perform a dual action: to have antibiotic drug activity and to be able to activate innate bactericidal mechanisms.

Despite extensive research into particle-engineering techniques for both dry powder and liquid formulations employing various [9–11,13,18,19], no commercial aerosolised anti-tubercular

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therapy is currently available. Misra et al. [8] suggests this may be related to hurdles such as difficulties in formulation development, unknown safety of inhaled excipients and production scalability. The current investigation focuses on pulmonary dry powder delivery as it offers shorter treatment times, enhanced patient compliance and device portability.

The aim of this research was to present a novel method whereby three anti-tubercular antibiotics could be effectively combined into an inhaled dry powder formulation for efficient treatment of TB-infected patients. Bypassing the oral route of administration would allow for reduced oral dosing and related side effects. In addition, this triple antibiotic dry powder may address aforementioned issues to effectively treat pulmonary TB in a timely manner. The micron-sized dry powder is produced in a single step spray-drying process without excipients and combines three first-line antibiotics for TB, as suggested by the WHO, (pyrazinamide (PYR):rifampicin (RIF):isoniazid (IZD) in a ratio of approximately 5:2:1) [1]. The physico-chemical characteristics and aerosol performance of this triple-DPI powder were characterised using various *in vitro* and analytical techniques.

2. Methods

2.1. Dry powder aerosol production

The combined antibiotic dry powder was produced by spray-drying. A Buchi-290 Mini spray-dryer was operated in a closed loop, connected in series with a Buchi-296 dehumidifier and Buchi B-295 inert-loop (all from Buchi Laboratories, Flawil, Switzerland), using nitrogen as the drying gas. For the triple antibiotic dry powder, the feed solution consisted of the three antibiotics – pyrazinamide (8 mg/mL), rifampicin (3 mg/mL) and isoniazid (1.5 mg/mL) (all antibiotics from Hangzhou ICH Imp & Exp Co. Ltd., Hangzhou, China) – in an ethanol:water (50:50 v/v) solution. The same concentrations and solvent mixture were used to individually spray-dry the three antibiotics for analytical comparison. Spray-drying was undertaken with the following settings: inlet temperature 60 °C, atomiser 40 mm (approximately 500 L/h), aspirator 100% (40 m^3/h) and feed rate 5% (2 mL/min). Powders were protected from light and moisture in a non-transparent dessicated container at 25 °C and used within 3 days.

2.2. Particle morphology

Particle surface morphology was characterised using a Zeiss Ultra Plus scanning electron microscope (Carl Zeiss, Oberkochen, Germany) at an acceleration voltage of 5 kV. The sample powder was placed onto carbon tape and sputter-coated with approximately 15 nm of gold (Emitech K550X) prior to imaging.

2.3. Particle sizing

Each single and triple antibiotic spray-dried formulation was analysed using laser diffraction with the associated software calculating particle size distributions by the Mie theory (Malvern Mastersizer 2000, Malvern Instruments Ltd., Worcestershire, UK) to give their respective volumetric median diameter and span – defined as the difference between the 10th and 90th percentile particle diameters, divided by the volumetric median diameter. The refractive index (RI) for pyrazinamide (1.577) was used for all measurements, as it composes more than 50% of the triple antibiotic powder. Furthermore, the RIs of rifampicin (1.613) and isoniazid (1.588) are similar.

Powder was loaded into a Scirocco 2000 dry powder feeder and dispersed in a 3.5 bar airstream. The dispersive air pressure (3.5 bar) was chosen by comparing median particle size over the minimum to maximum pressures (0.5–4.0 bar). Measurements were performed in triplicate.

2.4. Aerosol dispersion and drug quantification

Aerosol performance of the antibiotic microparticles was assessed using a multi-stage liquid impinger (MSLI) (Copley Scientific, Nottingham, UK) coupled with a USP throat. The first four MSLI stages were each filled with 20 mL of rinsing solvent (methanol:50 mM phosphate buffer pH3.0, 50:50 v/v), and the fifth stage fitted with a 0.2 μm glass filter (Pall Corporation, Surry Hills, Australia). To minimise evaporation of the solvent, the MSLI airflow was equilibrated to approximately 100 L/min when empty, then after filling with solvent readjusted to exactly 100 L/min within 5 s, using a GAST Rotary vein pump (Erweka GmbH, Heusenstamm, Germany) and calibrated flow meter (TSI 3063, TSI instruments Ltd., Buckinghamshire, UK). An airflow of 100 L/min represents the flow rate achievable by patients using a comfortable inspiratory effort with an Aeroliser® DPI device (Novartis, Mulgrave, Australia) [20]. Size 3 hydroxypropyl methylcellulose capsules (Capsugel, West Ryde, Australia) were filled with approximately 20 mg of the powder and actuated for 2.4 s using an Aeroliser® connected to the USP via a mouthpiece adapter.

After actuation, the device, capsule, throat and stages 1–4 of the MSLI were washed using varying amounts of rinsing solvent, specifically: 10 mL each for the throat, device and stage 5, and 5 mL each for the adaptor and capsule. Each sample was tested in triplicate.

Quantification of the dispersed drug was performed using high performance liquid chromatography (HPLC). The method used was adapted and modified from Calleri et al. [21]. The Shimadzu HPLC system comprised of a CBM-20A controller, LC-20AT pump, SPD-20A UV/VIS detector, SIL-20A HT autosampler and LCSolution software (all from Shimadzu Corporation, Kyoto, Japan). It was coupled with a μBondapak™ C18 (3.9 × 300 mm) column (Waters, Milford, MA, USA) with a sample injection volume of 100 μL.

The mobile phases were 50 mM phosphate buffer (pH 3.0) (orthophosphoric acid from Ajax Finechem Pty. Ltd., Taren Point, Australia; potassium dihydrogen orthophosphate from Biolab Ltd., Clayton, Australia) (A), acetonitrile (B) and methanol (solvents both from V.S.Chem House, Bangkok, Thailand) (C). The gradient profile was A:C (9:1 v/v) for 5.25 min, followed by a simultaneous linear decrease to A:C (1:0 v/v) by 10.5 min and increase to A:B (50:50 v/v) by 14.5 min. The latter was maintained for 30 min and then a gradient initiated down to A:C (9:1 v/v) by 35 min, which was maintained until 40 min. The flow rate was kept at 1.0 mL min^{-1} throughout.

The UV wavelengths for detection were adjusted to detect the antibiotics microparticles at the following retention times: 261 nm initially for isoniazid at 3.7 min, then 265 nm from 5.25 min followed by 254 nm at 9.5 min, to detect pyrazinamide and rifampicin at 6.2 min and 22 min, respectively. Standards solutions were prepared for each of the three antibiotics at 0.01, 0.05, 0.1 and 0.2 mg/mL and gave an R^2 value of 1.00 for all antibiotics.

Total emitted dose describes the post-aerosolisation weight difference between the initial weight of drug loaded into the capsule and the amount retained within the Aeroliser®. The mass median aerodynamic diameter (MMAD) was obtained from the log-probability plot of the MSLI results. Fine particle fraction (FPF) was defined as the percent mass of aerosol particles with an aerodynamic diameter less than 5 μm.

2.5. Qualitative surface analysis

Time-of-Flight Secondary Ion Mass Spectrometry (ToF-SIMS) was used to image spectral data (surface chemical mapping based on mass fragment analysis) of the sample powder to provide