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Short Communication

Murine pharmacokinetics of rifapentine delivered as an inhalable dry powder

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ABSTRACT

A novel inhalable rifapentine dry powder formulation could improve pulmonary rifapentine concentrations resulting in a significantly shorter time to treat tuberculosis infection. The pharmacokinetics of rifapentine (20 mg/kg) in healthy mice was compared following intratracheal (IT) and intraperitoneal (IP) administration. Plasma, bronchoalveolar lavage (BAL) and tissue samples were collected and drug levels were quantified at time points up to 24 h. Concentration–time data were analysed using a mixed-effects modelling approach to provide model-based estimates of area under the concentration–time curve from time 0 to infinity ($AUC_{0-\infty}$). IT delivery had considerably higher peak rifapentine lung and BAL concentrations and associated $AUC_{0-\infty}$ compared with IP delivery. The plasma $AUC_{0-\infty}$ following IT dry powder delivery was ca. four-fold smaller than the value for IP delivery. Inhaled delivery of rifapentine has the potential to selectively enhance therapeutic efficacy at the pulmonary site of infection whilst minimising systemic exposure and related toxicity.

1. Introduction

One-third of the global population is infected with *Mycobacterium tuberculosis*, with the majority of people retaining latent infection and the lifelong risk of progressing to active, symptomatic disease [1]. Shorter treatment regimens for latent tuberculosis infection (LTBI) are urgently needed to improve patient adherence and cost effectiveness compared to the current 9-month oral isoniazid therapy [2]. Although daily oral rifapentine-based regimens resolved LTBI in an infected murine model in just 6–8 weeks [3], extrapolation of these results to patients is uncertain. Specifically, whilst oral rifapentine also shortened treatment duration in murine models of active tuberculosis (TB) infection, this was not reproduced in a subsequent human clinical trial [4]. The proposed explanations are related to the oral route of administration: (i) bioavailability of rifapentine is highly influenced by the concomitant ingestion of food, which was not well controlled in the study; and (ii) high plasma protein binding of oral rifapentine limited the attainment of effective concentrations within infected pulmonary granulomas.

The current work describes the delivery of an inhalable excipient-free rifapentine dry powder formulation designed to maximise antibiotic exposure at the primary site of infection in the lungs and to overcome limitations associated with oral antibiotic administration [5]. In anticipation of studies in a murine model of LTBI, the current study investigates the pharmacokinetics of equivalent rifapentine doses delivered by

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the intraperitoneal (IP) and intratracheal (IT) routes to healthy mice.

2. Materials and methods

2.1. Pharmacokinetic studies

Female BALB/c mice (6–8 weeks old) were obtained from the Australian Resources Centre (Perth, WA, Australia) and were housed under specific pathogen-free conditions in the animal facility at the Centenary Institute (Camperdown, NSW, Australia). All murine experiments were conducted with approval of the University of Sydney Animal Ethics Committee (Sydney, NSW, Australia).

2.2. Intraperitoneal and intratracheal delivery of rifapentine

A rifapentine dose of 20 mg/kg was used in this study based on well-tolerated oral doses to mice and humans [6,7]. Rifapentine (Hangzhou ICH Imp & Exp Co. Ltd., Hangzhou, China) was solubilised in dimethyl sulphoxide (DMSO) and then diluted with sterile phosphate-buffered saline (PBS) to give a final drug concentration of 1 mg/mL in 10% (v/v) DMSO before IP injection (20 mg/kg). For IT delivery, an inhalable rifapentine dry powder was produced according to a method detailed by Chan et al. [5] and was administered (20 mg/kg) to mice by insufflation (Dry Powder Insufflator™ model DP-4M; Penn-Century Inc., Glenside, PA). The insufflator was weighed before and after insufflations to determine the exact drug amount delivered, which varied between 52% and 70% of the loaded dose.

2.3. Sample collection and analysis

Six animals were sacrificed by carbon dioxide asphyxiation at predetermined time points (0.5, 1, 2, 4, 8, 12 and 24 h after dose administration) and were immediately exsanguinated by cardiac puncture. Within each time point, blood from two sequential animals was pooled and collected into EDTA (ethylene diamine tetra-acetic acid) tubes to allow sufficient sample for analysis (total of three pooled samples per time point). Bronchoalveolar lavage (BAL) was performed using 1 mL of PBS and the fluid retrieved into an Eppendorf tube. The lungs were extracted before opening the peritoneal cavity to collect the liver, spleen and kidney. BAL and organ samples were not pooled. All samples were stored on ice.

Blood was centrifuged (Allegra® X-12R Centrifuge; Beckman Coulter, Pasadena, CA) to separate plasma and was then deproteinated with an equivalent volume of acetonitrile before centrifugation and collection of the supernatant. The lungs, liver, spleen and kidneys were homogenised (Polytron® PT 1200 Homogenizer; Kinematica AG, Lucerne, Switzerland) in triple deionised water (20%, w/v), followed by deproteination and processing using the same method as for plasma.

Samples were kept at 4 °C and were analysed by high-performance liquid chromatography (HPLC) (Shimadzu Corp., Kyoto, Japan) using a validated method with slight modifications [8]. The lower limits of quantification and detection of rifapentine were 100 ng/mL and 20 ng/mL. The intraday and interday variability (relative standard deviation) of the assay in plasma and tissues ($n = 6$) were <8%. Recoveries were 90.4 ± 3.0% (plasma), 100.9 ± 5.6% (BAL), 90.6 ± 1.4% (lungs), 91.4 ± 3.3% (liver), 90.6 ± 3.7% (kidney) and 97.7 ± 6.2% (spleen).

2.4. Pharmacokinetic analysis

The maximum rifapentine concentration (C_{max}) and the associated time (T_{max}) were determined by direct observation of the data. Concentration data were normalised for animal weight and total administered rifapentine dose.

2.4.1. Population pharmacokinetics

A mixed-effects modelling approach was employed to provide model-based estimates (and interanimal variability) of area under the concentration–time curve from time 0 to infinity ($AUC_{0-\infty}$) using ADAPT 5 software [9]. As destructive sampling was used in the conduct of this study, only one concentration–time sample per animal was available to be included in the analysis. The pharmacokinetics of rifapentine were characterised by fitting candidate population pharmacokinetic models to the data, using a maximum likelihood estimation method available in ADAPT 5 [9]. The focus of the analysis was to generate a robust estimate of clearance (CL), which was used to calculate $AUC_{0-\infty}$ based on the formula ($AUC_{0-\infty}$ = Dose/CL). Model robustness was supported by estimates of precision and accuracy for each parameter mean value (Supplementary Table S1).

To strengthen the pharmacokinetic estimates and to set realistic limitations on the calculation of the parameters, three 'subjects' with extensive data were included. In total, the number of 'subjects' following IT administration with plasma observations was 24, whilst all other matrices consisted of 45 mice. Following IP administration, the number of subjects with plasma observations was 21, whilst all other matrices consisted of 39 mice. The interanimal variability of key pharmacokinetic parameters for the IT route [standard deviation (SD) as the percent coefficient of variation (CV%)] ranged from 28.4% (AUC, kidney) to 40.6% (AUC, liver), whilst variability in the IP route ranged from 15.5% (AUC, liver) to 34.2% (AUC, lung), respectively. Supplementary Table S1 contains the mean parameter estimates, relative standard error and CV% for each organ following the respective routes of administration.

2.4.2. Residual variance models

Residual variance models were tested for rifapentine. The empirical variance models assumed that the random errors in measurements of concentrations of rifapentine in plasma were similar for all of the animals in the study and that the residual (error) SDs of the observations (σ) were linearly related to the true values (Y): $\sigma = SD_{slope}\, Y + SD_{intercept}$, in which SD_{slope} and $SD_{intercept}$ are the variance parameters. The initial empirical estimates for the variance parameters were based on the assay performance as described above.

3. Results

3.1. Rifapentine pharmacokinetics

The plasma, BAL fluid and tissue drug concentration–time profiles and the pharmacokinetic parameter data following IT and IP administration of rifapentine are presented in Fig. 1 and Tables 1 and 2, respectively. Drug solution was visible in the peritoneum of mice receiving IP rifapentine at the 0.5-h time point owing to insufficient time for complete peritoneal absorption. Therefore, the initial sampling time point for the IP delivery route data was set at 1 h.

For both routes of administration (IT and IP), plasma drug concentrations (Fig. 1A) rose to a peak (C_{max}, 8.4 ± 4.2 μg/mL and 15.8 ± 1.4 μg/mL, respectively) at 12 h, followed by a decrease to 24 h (6.5 ± 1.3 μg/mL and 13.5 ± 0.8 μg/mL, respectively). Table 1 shows that the plasma $AUC_{0-\infty}$ after IT dry powder delivery (455.1 mg/L h) was ca. four-fold smaller than the IP value (2010.1 mg/L h).

Following IT delivery, the maximum rifapentine concentration (25.2 ± 6.4 μg/mL) in BAL fluid (Fig. 1B) was identified at 0.5 h, reducing to 1.9 ± 0.1 μg/mL at 24 h after dosing. This was associated

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