

High-dose inhaled rifampicin powder formulations: preparation, *in vitro* characterization and *in vivo* evaluation

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Summary

Despite several studies on inhaled rifampicin in the literature, there have been no reports on the *in vivo* safety and pharmacokinetics of high-dose (>20 mg/kg) inhaled rifampicin. A high-dose of rifampicin is necessary to achieve drug high concentration in the lungs and the systemic circulation to treat both pulmonary and extra-pulmonary TB. While the use of high-dose of rifampicin from the oral route is associated with increased risk of toxicity including hepatotoxicity, the pulmonary delivery is an alternative approach to achieving higher drug concentration in the lungs and the systemic circulation with a lower dose than that from oral route. In this study, high-dose amorphous and crystalline powder formulations were prepared and characterized *in vitro*. Then, the safety and pharmacokinetics of rifampicin were studied after repeated administration to *Sprague Dawley* rats by intra-tracheal insufflation once daily for seven days. Among the powder formulations prepared, the amorphous and the crystalline dihydrate formulation showed better aerosolization stability compared to the crystalline pentahydrate formulations and were selected for further *in vivo* evaluations. Repeated intra-tracheal administration of high-dose rifampicin powder formulations (50 mg/kg) were well tolerated by laboratory rats and were safe to the lungs and the liver. The intra-tracheal administration of rifampicin achieved significantly higher area under the plasma concentration-time curve (AUC) compared to that from oral rifampicin at the same dose. Inhaled administration of high-dose rifampicin, therefore, has the potential to achieve higher systemic bioavailability than oral rifampicin and can be beneficial in improving TB treatment.

Key Message

Intra-tracheal administration of rifampicin results in significantly higher systemic drug bioavailability compared to the oral rifampicin at the same dose suggesting the potential of inhaled rifampicin in achieving better therapeutic effects in TB treatment.

Introduction

Rifampicin is one of the potent, highly effective first-line drugs for the treatment of Tuberculosis (TB). The maximum recommended dose of rifampicin for TB treatment is 10 mg/kg per day from the oral route [1]. While doses higher than the recommended dose are found to be more effective in clinical studies [2], higher oral doses of rifampicin have not been approved for therapeutic use. The use of high doses of rifampicin via the oral route increases the risk of systemic toxicity of rifampicin, mainly the risk of rifampicin induced hepatotoxicity increases with chronic use of high doses.

During TB infection, *Mycobacterium tuberculosis* mainly localize in the lungs, known as pulmonary TB. This is characterized by the presence of bacteria within the granulomatous lesions in the lungs that are devoid of vasculature. TB infection also spreads to and affects other organs termed extra-pulmonary TB. A high concentration of a drug is therefore necessary in both the lungs and the systemic circulation for an efficient bactericidal effect. Inhaled delivery of rifampicin is an alternative approach to deliver a high concentration of the drug to the lungs as well as the systemic circulation at a dose lower than that from the oral route. A higher systemic concentration of rifampicin can be achieved due to the absence of hepatic first pass metabolism from the pulmonary route. Moreover, pulmonary administration allows delivery of higher drug concentrations to the vicinity of lung lesions and facilitates effective penetration of the drug into the lesions where highly sequestered mycobacteria reside [3].

Several studies on inhaled rifampicin formulations and their *in vitro* and *in vivo* behaviour have been reported in the literature. However, no study has reported high-dose rifampicin formulations for inhalation, which are clinically relevant for TB treatment. Moreover, there have been very few studies to compare the oral and inhaled pharmacokinetics of high-dose rifampicin. Studies reported in the literature either evaluated a low dose (≤ 20 mg/kg) inhaled rifampicin or the formulation reported contained excipients suggesting a low payload formulation. Similarly, there were no reports on the pharmacokinetics of rifampicin after repeated inhaled administration, prior to this study. For inhaled rifampicin therapy to be a part of the anti-TB regimen, its documented *in vivo* respiratory tract safety, together with a demonstration of its potential to achieve higher bioavailability than the oral route, is necessary.

This study aimed to investigate the *in vivo* safety and pharmacokinetics of inhaled high-dose rifampicin in comparison to oral rifampicin in laboratory rats. The formulations tested were amorphous and crystalline high-dose powder formulations of rifampicin to investigate if they resulted different *in vivo* effects.

Experimental methods

Materials: The following materials were purchased: British Pharmacopoeia grade Rifampicin from Hangzhou Dayangchem Co., Ltd. (Hangzhouzhejiang, China); phosphate buffered saline (PBS) tablets from Fisher scientific New Zealand; paraformaldehyde and sucrose ($\geq 99.5\%$) from Sigma-Aldrich (St. Louis, USA); alanine transaminase (ALT) and aspartate aminotransferase (AST) activity assay kits from Abcam, Cambridge, United Kingdom; paraformaldehyde, sucrose ($\geq 99.5\%$), and mass spectrometry grade ammonium formate and formic acid from Sigma-Aldrich (St. Louis, USA); and high-performance liquid chromatography (HPLC) grade solvents from Merck, Darmstadt, Germany.

Preparation of rifampicin powder formulations: Inhalable rifampicin powder formulations were prepared by spray drying and crystallization methods. Spray drying was performed at an inlet temperature of 90°C using a Buchi B-290 Mini Spray-Dryer (Buchi Labortechnik AG, Flawil, Switzerland) in a closed-mode. Rifampicin solution (0.5% w/v) in ethanol:water (90:10 v/v), was the feed solution for the spray drying method. For crystallization, ethanol was used as the crystallization solvent. Raw rifampicin was added to ethanol, and the mixture was exposed to ultrasonic waves. Crystallization of rifampicin particles occurred by solid-state transformation of the raw rifampicin. The resulting suspensions were centrifuged at 2000 rpm to obtain the particles. Powder formulations were obtained by drying the sediment in an oven at 60°C for 48 h.

Particle sizing and crystallinity: Particle sizing was performed by laser light diffraction technique (Horiba LA-950 particle size analyzer, Japan). Crystallinity was determined by X-ray powder diffraction (XRPD).

In vitro aerosolization: *In vitro* aerosolization performance was studied using a Next Generation Impactor. Powders (20 mg) were actuated using Aerolizer[®] at 100 L/min for 4 seconds. The fine particle fraction (FPF) was calculated as percentage fraction of particles with cut-off diameters ≤ 5 μm , in recovered dose.

Animals and ethical considerations: Male Sprague Dawley Rats (275 to 325 g) were obtained from the Hercus Taieri Resource Unit, University of Otago, Dunedin, New Zealand. The study was approved by the Animal Ethics Committee at the University of Otago (Protocol number AUP-18-199).

Administration of rifampicin powder formulations: Intra-tracheal powder insufflation of 25 mg/kg or 50 mg/kg rifampicin was performed using a dry powder insufflator. The efficiency of the insufflation was between 92.8% and 96.7% depending on the powder formulation. For oral administration, rifampicin powder (50 mg/kg), suspended in normal saline, was administered by oral gavage using a feeding tube connected to a syringe.

Blood sampling, euthanasia and tissue collection: For the pharmacokinetic study, blood samples were collected from cannulated jugular vein at different time points for 24 h after drug administration on Day 0 and Day 6. Rats were euthanized 24 h after final drug administration (Day 7), and the lungs and blood samples were collected.

Liver enzymatic activity assay: Alanine transaminase (ALT) activity was measured in serum using ALT assay kits following manufacturer's protocol.

Histopathology of lungs: Lung tissues were frozen at -20°C in optimal cutting temperature compound, and thin sections (8 to 10 μm in thickness) were prepared by cryo-sectioning using a Leica Cryostat Microtome (Leica Biosystems, Germany). The sections were mounted on superfrost glass slides and stained by Hematoxylin and Eosin (H&E) stain. Images were captured using a slide scanner (Leica Biosystems Nussloch GmbH).

Quantification of rifampicin in plasma and tissues: Rifampicin quantification in biological samples (rat plasma and lung tissue homogenates) was performed using liquid chromatography tandem mass spectrometry (LC-MS/MS) analysis in positive ionization mode. The stationary phase used was a Kinetex EVO C18 column (5 μm , 2.1 X 100 mm; Phenomenex, California, USA) with the mobile phase comprising of 40:60 (v/v) acetonitrile (containing 0.05% formic acid) and 10 mM ammonium formate in water. Linearity of the assay was verified for a concentration range of 5 ng/mL to 240 ng/mL with the bias and precision within the specified limits ($\leq 15\%$).

Statistical analysis: Data represent mean $\pm$ standard deviation (SD). Statistical comparison was performed by one-way analysis of variance (ANOVA) and Student-Newman-Keuls post-hoc tests were using GraphPad Prism 5 software (GraphPad Software, San Diego, USA). Differences were considered significant when p values were less than 0.05.

Results and discussion

The powder formulations prepared were amorphous (RIF A), crystalline pentahydrate (RIF C1 and RIF C2) and crystalline dihydrate (RIF C3) formulations of rifampicin (**Figure 1**) as determined by XRPD. All powder formulations had mean particle size $<5\ \mu\text{m}$, emitted dose (ED) greater than 75% and FPF greater than 50% [4].

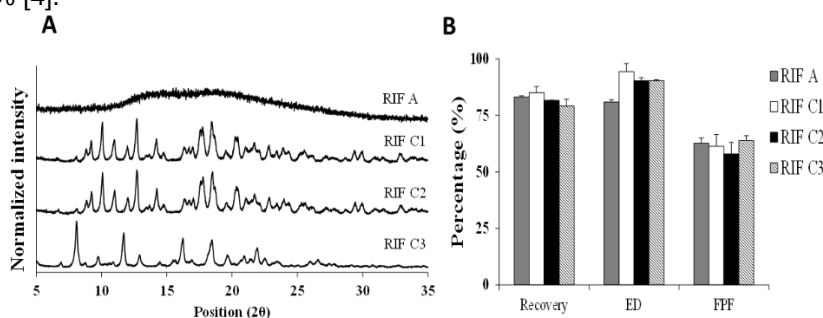


Figure 1 - X-ray diffraction patterns of rifampicin powder formulations (A); and the *in vitro* aerosolization parameters (B).

The serum alanine transaminase (ALT) activity assay showed significantly higher ALT activity, compared to the control group, after single and repeated oral administration and after repeated intra-tracheal administration of 50 mg/kg RIF A (**Figure 2**). In the case of RIF C3, significantly higher ALT activity was observed also in the repeated intra-tracheal 25 mg/kg group. The results suggested a lesser drug burden on the liver after intra-tracheal administration of rifampicin compared to its oral administration.

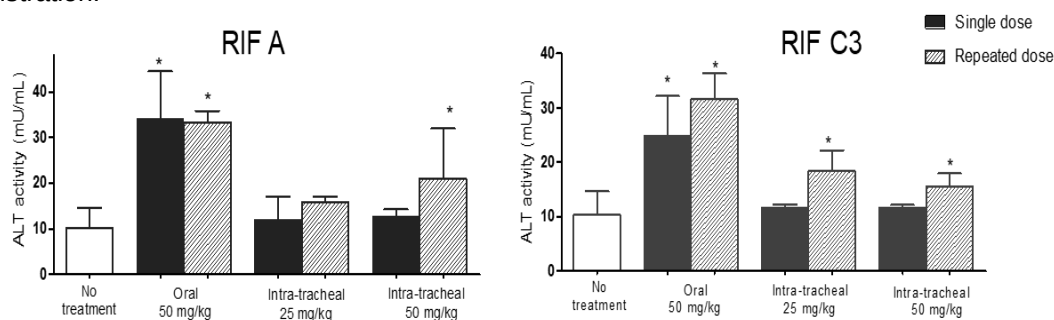


Figure 2 - Serum alanine transaminase (ALT) activity measured 24 h after single and repeated administration of RIF A and RIF C3 powder formulations in rats. Asterisk represents significant difference compared to the non-treated control group.

The histopathological evaluation suggested no toxicological changes in rat lung sections after intra-tracheal administration of up to 50 mg/kg rifampicin powder formulations for seven days. Although mild bronchial changes and epithelial thickening were observed in the intra-tracheal administration group (as shown by red arrows in **Figure 3**), they were not due to rifampicin toxicity (as indicated by absence of lymphocyte and eosinophil infiltration, alveolar septal thickening, macrophage accumulation or lesions) but a response to foreign particle inhalation by sensitive airways of rats [5].

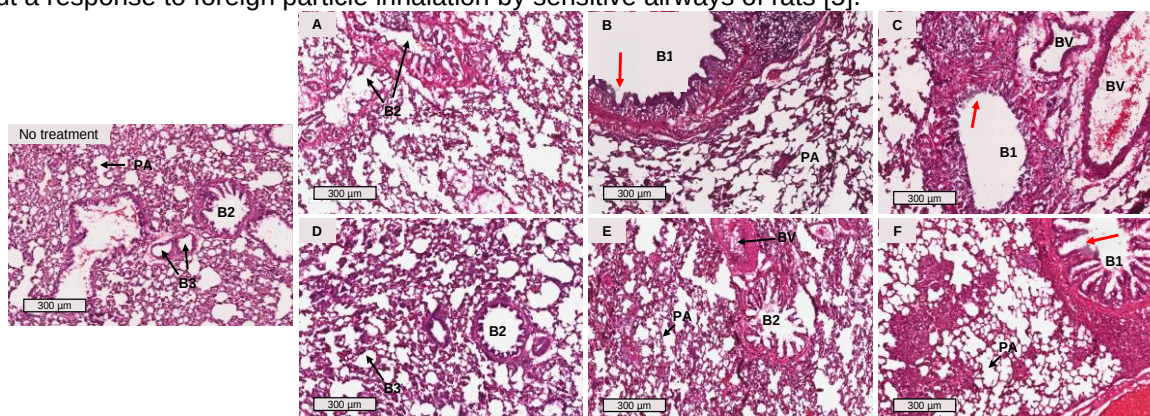


Figure 3 - Representative images showing hematoxylin and eosin (H&E) stained histological sections of rat lung after no treatment (control) and after seven repeated administration of RIF A oral 50 mg/kg (A); RIF A intra-tracheal 25 mg/kg (B); RIF A intra-tracheal 50 mg/kg (C); RIF C3 oral 50 mg/kg (D); RIF C3 intra-tracheal 25 mg/kg (E); and RIF C3 intra-tracheal 50 mg/kg (F). (Scale bar: 300 µm, Magnification: 8X). PA: Pulmonary alveoli; BV: Blood vessel; B1: Bronchi; B2: Bronchiole; B3: Bronchioli.

The plasma-concentration time profiles of rifampicin showed significantly higher plasma concentrations from the intra-tracheal 50 mg/kg group compared to the oral rifampicin at the same dose (**Figure 4**). After administration of seven repeated doses of rifampicin, there was an accumulation of rifampicin in the systemic circulation, as shown by increased plasma concentrations on Day 6 (**Figure 4B**). This suggested that enzyme auto-induction behaviour of rifampicin was not observed in this study, which might be because of the high-dose used or because the enzyme auto-induction phenomenon had not started by Day 6. The effects after administration of RIF C3 was similar in which the intra-tracheal administration led to significantly higher plasma concentrations compared to the oral administration at the same dose (**Figure 5**). However, the plasma concentrations were lower compared to that from RIF A suggesting lower systemic bioavailability of rifampicin from the crystalline formulation after both oral and intra-tracheal administration. Repeated administration of RIF C3 showed increased plasma concentration of rifampicin compared to that after a single dose (**Figure 5B**), suggesting absence of enzyme auto-induction behaviour also in the case of the crystalline formulation. For both formulations, there was no significant difference in the lung tissue concentration of rifampicin at 2 hours after oral and intra-tracheal administration of 50 mg/kg rifampicin.

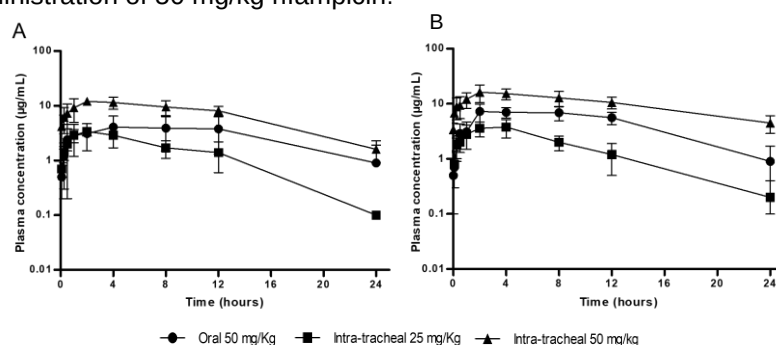


Figure 4 - Average log scale plasma concentration–time profiles of rifampicin after single (A) and repeated (B) administration of amorphous powder formulation (RIF A) by oral (50 mg/kg) and intra-tracheal (25 and 50 mg/kg) administration to Sprague Dawley rats.

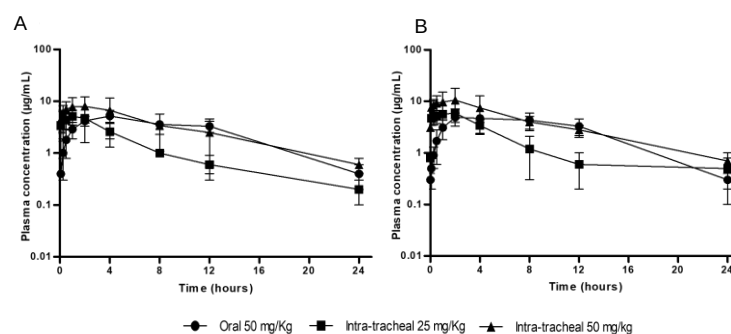


Figure 5 - Average log scale plasma concentration–time profiles of rifampicin after single (A) and repeated (B) administration of crystalline powder formulation (RIF C3) by oral (50 mg/kg) and intra-tracheal (25 and 50 mg/kg) administration to *Sprague Dawley* rats.

Conclusions

The results from ALT activity assay suggested that intra-tracheal administration of rifampicin leads to reduced drug burden on the liver compared to oral rifampicin by avoiding hepatic first pass metabolism after intra-tracheal administration. The histopathological evaluation suggested that both amorphous and crystalline powder formulations of rifampicin were safe to rat lungs after seven repeated administration of up to 50 mg/kg rifampicin. The plasma concentration-time profiles indicated that intra-tracheal rifampicin can achieve significantly higher systemic bioavailability compared to oral rifampicin at the same dose. Amorphous rifampicin was preferable to the crystalline rifampicin formulation due to its higher systemic bioavailability. These findings are encouraging for further pre-clinical and clinical development of high-dose rifampicin powder formulations for inhaled anti-TB therapy.

References

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