Surface Modified Voriconazole Dry Powder Inhalable Formulation for the Treatment of Invasive Pulmonary Aspergillosis

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Summary

Background: Invasive pulmonary aspergillosis (IPA) is a severe disease in immunocompromised patients with extremely high mortality rate. Voriconazole (VRZ) is a first line treatment drug for IPA, conventionally administered orally or intravenous, resulting in a plethora of drug-drug interactions and off-target toxic effects. In the present research work, we developed and characterised a highly dispersible dry powder inhalable formulation of VRZ using L-Leucine as a dispersibility enhancer. **Methods:** VRZ and L-Leucine in varying concentrations were dissolved in ethanol-water (70:30% v/v) and spray dried to yield inhalable dry powders. Powders were characterised in terms of particle size, morphology and aerosol performance using the low resistance RS01 dry powder device with next generation cascade impactor. Storage stability (chemical stability and aerosol performance) of the optimized formulation was evaluated for 3 months. Calu-3 sub bronchial epithelial cell line was used to study cell viability (MTS test). Finally, *in vivo* pharmacokinetic studies in mice were carried out to determine the lung bioavailability of the optimised formulation. **Results:** Dry powder comprising VRZ (8 mg/mL) and L-Leucine (2 mg/mL) was found to be suitable for inhalation therapy. Powder exhibited a volume median diameter of 2.64 ± 0.05 µm and superior aerosolisation with MMAD of 3.79 ± 0.02 µm and fine particle fraction (% aerosol < 5 µm) of 60.00 ± 0.94 %. Powder exhibited irregular morphology and demonstrated physico-chemical stability of up to 3 months at room temperature. Formulation was found to be non-cytotoxic to Calu-3 cells. Moreover, lung bioavailability in murine model showed the ability of inhaled formulation to attain higher concentration of VRZ in lungs as compared to intravenous administration. **Conclusion:** A highly respirable dry powder VRZ formulation was developed for the treatment of IPA.

Introduction

Aspergillus fumigatus, the opportunistic fungi, causes IPA particularly in immunocompromised patients such as those suffering from hematologic malignancies, cancer, AIDS and those undergoing solid organ transplantation.[1] This results in substantial mortality (nearly 80%) and huge financial burden. VRZ is the drug of choice for the treatment of IPA.[2] Oral or intravenous administration of VRZ have been associated with high inter- and intra-patient pharmacokinetic variability, poor lung distribution particularly in patients undergoing lung transplantation, alteration of enzyme levels in liver leading to numerous, sometimes lethal drug-drug interactions as well as the off-target toxic effects. [3]

Pulmonary delivery of high doses of VRZ represent a potential viable therapeutic option for the targeted treatment of IPA, whilst minimising systemic exposure and related toxicity.

Methods and Materials

VRZ was supplied by Ranbaxy Laboratories (Gurgaon, India) and L-Leucine was purchased from Sigma-Aldrich (Sydney, Australia). Calu-3 cell line (HTB-55) was purchased from the American Type Cell Culture Collection (ATTC, Rockville, USA). Dulbecco's modified Eagle's medium and L-glutamine from Invitrogen (Sydney, Australia). All solvents were of analytical grade and used as supplied (Biolab, Victoria, Australia)

Preparation of L-Leucine modified VRZ microparticles

For the preparation of respirable particles, VRZ (8 mg/mL) and L-Leucine (2 mg/mL) were dissolved in ethanol-water (70:30% v/v) and spray dried using a Buchi Mini Spray Dryer B-290 at the following conditions: feed concentration of 10 mg/ml, inlet temperature 125°C, outlet temperature was 78°C, atomiser 700 L/h, aspirator 40 m3/h and feed rate 5%.

Morphological and Particle Size Analysis

Morphology of the spray dried products was studied using a scanning electron microscope (SEM, JMC, 6000 JEOL, Japan). Samples were coated with 15 nm gold (Sputter coater S150B, Edwards High Vacuum, Sussex, UK) and images were taken at random locations. Size distribution of the VRZ alone and VRZ-Leucine particles was analysed using laser light diffraction (Mastersizer 3000, Malvern, United Kingdom) using the Scirocco dry dispersion unit with a feed pressure of 4 bar and a refractive index of 1.62 for VRZ.

***In vitro* aerosol performance characterisation**

Aerosol performance of the spray dried products (5mg in a size 3 gelatin capsule) was evaluated using an RS01 dry powder inhaler device (Plastiape, Italy) with a next generation impactor (NGI) operated at a flow rate of 60 L/min for 4 sec. Under these operating conditions, the volume of air drawn through the inhaler corresponds to 4 L, which represent the normal inspiratory capacity of an average sized-adult male of 70 kg. Samples were recovered from each stage of the NGI and the VRZ content was determined by a validated HPLC method. Mass median aerodynamic diameter (MMAD), geometric standard deviation (GSD) and fine particle fraction (FPF) (% aerosol < 5 µm) of the emitted dose were calculated from the NGI results.

Short Term Storage Stability

Storage stability of optimised formulation was determined as per USFDA guidelines.[4] Optimised formulation was stored under two conditions: Condition 1: 25ºC and 60% RH and Condition 2: 40ºC and 75% RH in climate controlled cabinet and assessed for their chemical stability and aerosol performance for up to a 3 months.

Calu-3 cell viability

Calu-3 cell viability for the spray dried VRZ only and L-Leucine modified VRZ was carried out according to the previous published method.[5] Briefly, cells were seeded at the density of 5×10^4 cells/well, incubated overnight and treated with the increasing equivalent concentrations of VRZ (1.2 nM to 300 µM) for both the spray dried products for 72 h. 20 µL of the CellTiter 96® Aqueous assay (MTS reagent) (Promega, Madison, USA) was added to each well to assess the viability of the cells. The plates were incubated for 3 hours at 37°C in humidified 5% CO_2 atmosphere. The absorbance was measured at 490 nm using a Wallac 1420 VICTOR 2 Multilabel Counter (Wallac, Waltham, USA).

***In vivo* lung bioavailability**

Animal experimentation were carried out after obtaining ethical clearances from the Institutional Animal Ethics Committee of the National Institute of Pharmaceutical Education and Research (NIPER), S.A.S Nagar India. Balb/c mice of either sex (20-25 g) were divided in two groups: Group 1 (40 animals) were dosed with optimised inhalable formulation (target VRZ dose 10 mg/kg) using a custom built in house apparatus while Group 2 (40 animals) received an intravenous VRZ dose (10mg/kg). At predetermined time points (10 min, 30 min, 1, 2, 4, 8, 12 and 24 h), five mice were euthanised with pentobarbital injection. Whole blood was collected following cardiac puncture and lungs were also excised and stored at -20ºC until further analysis. VRZ was quantified by validated HPLC method following homogenisation of lung tissue according Beinborn et al protocol with minor modifications.[6]

Results and Discussion

Dry powder formulation containing VRZ (8 mg/mL) and L-Leucine (2 mg/mL) was found to have optimum characteristics for inhalation therapy. Figure 1 shows the representative scanning electron microscopy images of spray dried VRZ alone and optimised L-Leucine modified VRZ microparticles (VRZ_LEU_20). Spray dried VRZ exhibited irregular plate like morphology with crystalline structure. However, with the inclusion of L-Leucine in the spray drying feed, the morphology of composite particles were found to be more regular and spherical. Particle size analysis by laser diffraction indicated median volume diameters ($dv_{0.5}$) of 4.52 ± 0.07 µm and 2.64 ± 0.05 (n=3) for VRZ alone and VRZ_LEU_20, respectively.

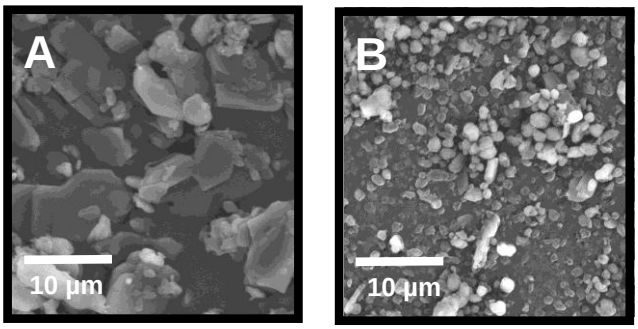

Figure 1 Representative scanning electron microscopy images (A) Spray dried VRZ alone and (B) VRZ_LEU_20

The *in vitro* aerosolisation performance of the spray dried VRZ alone and VRZ_LEU_20 is shown in Figure 2. The MMAD and FPF (% aerosol < 5µm) of VRZ alone was found to be 6.12 ± 0.18 µm and 20.86 ± 1.98 %, respectively, while for VRZ_LEU_20, it was found to be 3.79 ± 0.02 µm and 60.00 ± 0.94 %, respectively. Incorporation of L-Leucine clearly lead to an improvement ($p<0.05$) of the aerosolisation performance of the spray dried composite particles. L-Leucine probably increased aerosol performance by reducing particle agglomeration, thus promoting particle deagglomeration and delivery.[7]

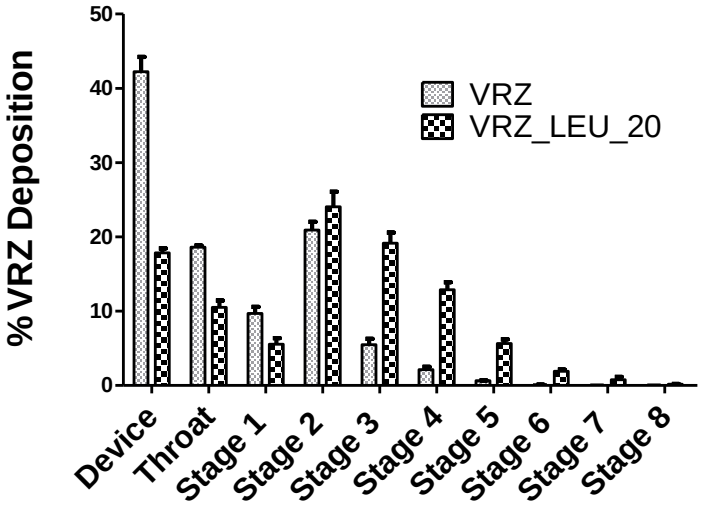

Figure 2 Aerodynamic particle size distribution profile of VRZ and VRZ_LEU_20 with NGI at a flow rate of 60 L/min. For each stage, VRZ is shown as a percentage of its total actual recovered amount. (n=3; mean ± SD)

The optimised formulation (VRZ_LEU_20) was found to be chemically stable in terms when stored for 3 months at room temperature as well as accelerated storage conditions. No significant change ($p>0.05$) in the aerosol performance of VRZ_LEU_20 was observed when powders were stored at 25ºC and 60% RH for three months. However, nearly 10% decrease in FPF (% aerosol < 5µm) of VRZ_LEU_20 was observed when it was stored at 40ºC and 75% RH. This clearly revealed that the optimised formulation should be protected from high humidity and high temperature conditions for its optimal performance.

The dose response cytotoxicity profile of spray dried VRZ alone and VRZ_LEU_20 on Calu-3 cells is shown in Figure 3. Calu-3 cells could tolerate (nearly 90% cell viability) a wide range of VRZ concentrations, from 1.2 nM to 300 µM indicating that it can be safely administered to the lungs in this range.

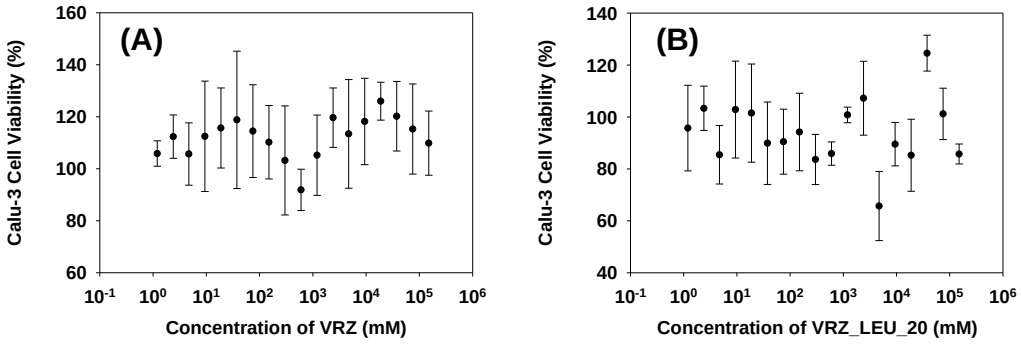

Figure 3 The effect of VRZ (A) and VRZ_LEU_20 (B) on Calu-3 Cell viability following 72 h VRZ treatment. (n=3; mean ± SD)

Figure 4 shows the plasma and lung VRZ concentration time profiles following intravenous administration of VRZ solution and inhalation delivery of optimised formulation (VRZ_LEU_20). *In vivo* lung bioavailability studies in murine model suggested that inhalable VRZ formulation (VRZ_LEU_20) was able to reach higher VRZ concentrations in the lungs compared to intravenous administration, thereby, enhancing the therapeutic effect of the drug at the disease site. Total lung VRZ exposure $AUC_{0-\infty}$ was found to be 524.31 ± 170.05 mg/kg h wet lung weight and 32.89 ± 9.95 mg/kg h wet lung weight when administered through inhalation and intravenous delivery, respectively. Similarly, C_{max} in the lungs was found to be 1095.25 ± 277.92 µg/g and 13.48 ± 5.35 µg/g when VRZ was administered through inhalation and intravenous route of administration, respectively.

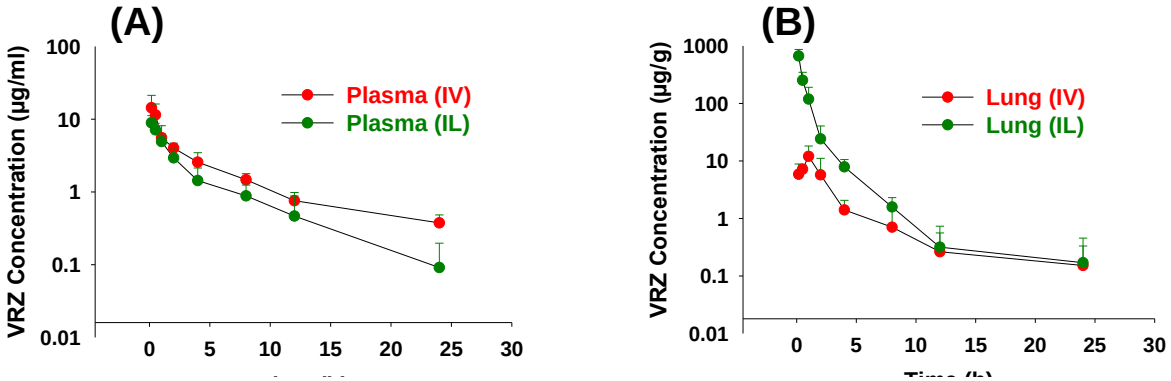

Figure 4 Voriconazole (VRZ) concentration–time plots following intravenous (IV) and inhalation (IL) delivery (mean ± standard deviation) (n = 5) for (A) Plasma and (B) Lung.

Conclusions

IPA is a serious disease in immunocompromised patients with unmet medical needs. Pulmonary delivery of high dose of VRZ could serve as attractive therapeutic alternative for the treatment of IPA. The present study confirmed the suitability of L-Leucine modified VRZ formulation for the inhalation therapy. The formulation was found to be high dispersible, stable for 3 months under room temperature conditions and non-toxic to the pulmonary epithelial cells. In addition, murine pharmacokinetics studies revealed that inhalable VRZ formulation can achieve higher concentrations of VRZ in the lungs as compared to conventional intravenous administration, thereby, may lead to better therapeutic outcome.

Acknowledgments

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References

1. Patterson, T. F: *Advances and challenges in management of invasive mycoses*. Lancet 2005; 366: pp1013-1025.

2. T.J. Walsh, E.J. Anaissie, D.W. Denning, R. Herbrecht, D.P. Kontoyiannis, K.A. Marr, V.A. Morrison, B.H. Segal, W.J. Steinbach, D.A. Stevens, J.A. van Burik, J.R. Wingard, T.F. Patterson, A: *Infectious Diseases Society of, Treatment of aspergillosis: clinical practice guidelines of the Infectious Diseases Society of America*, Clin Infect Dis 2008; 46: pp 327-360.

3. Hilberg, O., Andersen, C. U., Henning, O., Lundby, T., Mortensen, J., Bendstrup, E: *Remarkably efficient inhaled antifungal monotherapy for invasive pulmonary aspergillosis*. Eur Respir J 2012; 40: pp 271-273.

4. F. Draft, *Guidance for industry—metered dose inhaler (MDI) and dry powder inhaler (DPI) drug products, Chemistry, manufacturing, and controls documentation* Oct. 1998.

5. Haghi, M., Young, P. M., Traini, D., Jaiswal, R., Gong, J., Bebawy, M: *Time- and passage-dependent characteristics of a Calu-3 respiratory epithelial cell model*. Drug Dev. Ind. Pharm. 2010; 36: pp 1207-1214.

6. N.A. Beinborn, J. Du, N.P. Wiederhold, H.D. Smyth, R.O. Williams, 3rd: *Dry powder insufflation of crystalline and amorphous voriconazole formulations produced by thin film freezing to mice.* Eur J Pharm Biopharm 2012; 81: pp 600-608

7. L. Cruz, E. Fattal, L. Tasso, G.C. Freitas, A.B. Carregaro, S.S. Guterres, A.R. Pohlmann, N. Tsapis: *Formulation and in vivo evaluation of sodium alendronate spray-dried microparticles intended for lung delivery*. J Control Release 2011; 152: pp 370-375.