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Improved delivery of voriconazole to *Aspergillus fumigatus* through solid lipid nanoparticles as an effective carrier

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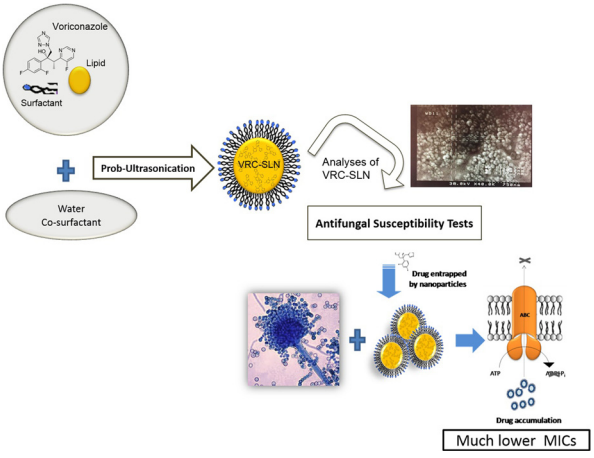
GRAPHICAL ABSTRACT



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ABSTRACT

Novel voriconazole-loaded solid lipid nanoparticles (VRC-SLNs) were prepared via probe-ultrasonication method, and the resultant nanoparticles were tested on *A. fumigatus*. Voriconazole-loaded solid lipid nanoparticles were prepared using the probe ultrasonication technique. Photon correlation spectroscopy (PCS) was used to determine the average particle size and zeta potential of SLN formulations. Transmission electron microscopy was also used to determine the morphology of solid lipid nanoparticles. To determine MIC for all SLN formulations against strains of *Aspergillus* the Clinical and Laboratory Standards Institute guidelines was followed. The results showed that SLNs containing voriconazole exhibited almost spherical shape with a diameter

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and zeta potential of 286.6 ± 4.7 nm and −15 ± 4.1 mV respectively. This novel formulation of VRC led to a significant reduction in MICs for all *Aspergillus* either VRC-susceptible or VRC-resistant isolates ($P < 0.05$). The MIC_{50} drug concentration was obtained as 0.015 μg/ml for both VRC-susceptible strains of *A. fumigates* while it was 0.25 μg/ml against VRC ($p < 0.05$). VRC-resistant strains showed a MIC_{50} of 0.015 μg/ml as well. These novel drug formulations may increase the bioavailability through an increase in the dissolution rate of voriconazole. This study showed, for the first time, VRC-SLNs can be employed as an effective delivery systems for VRC on *A. fumigatus* isolates.

1. Introduction

Azoles are the only class of oral antifungal available for the treatment of *Aspergillus* diseases.

Aspergillus is a saprophytic conidial mold isolated abundantly from soil, construction dust and hospitals [[1] [2]], which causes a broad spectrum of pulmonary aspergillosis (allergic bronchopulmonary aspergillosis (ABPA), chronic pulmonary aspergillosis (CPA) and invasive pulmonary aspergillosis (IPA)) [3].

Voriconazole (VRC) is used as the first-line treatment for invasive aspergillosis [4] and is available in oral and intravenous formulations. Voriconazole is a second-generation broad-spectrum triazole that inhibits the cytochrome P450-dependent enzyme lanosterol 14 alpha-demethylase, and subsequently, interrupts the essential step for ergosterol biosynthesis in the fungal cell [5]. Apart from the clinical implications of resistance, exposure of the fungus to azoles in the environment is the second route for induction of resistance [6]. In a recent international surveillance study, the prevalence rate of azole-resistant *A. fumigatus* isolates was determined to be 3.2% [7]. Moreover, it has been reported that the prevalence of azole-resistant *A. fumigatus* in Iran has gone up remarkably from 3.3% to 6.6% [8]. Therefore, designing new antifungal agents/formulations or novel drug delivery systems is highly required to introduce a new phase in the management of invasive aspergillosis.

Generally, solid lipid nanoparticles (SLNs) contains biodegradable lipid which could be in a solid form at both room and body temperatures and their particle size varies between 50 and 1000 nm [9,10]. They have numerous advantages such as drug protection against harsh environmental situations, ease of large scale production using high pressure homogenization technique, biocompatibility, and biodegradability [11]. This carrier can be administered through general routes, which can transport the drug to the target site. Biodegradable lipid matrix, high drug loading, increased drug stability, controlled drug release, and enhanced penetration of drugs into the skin or any other target are some of the other advantages of SLNs [12,13].

Therefore, the aim of the present study was to develop a new drug delivery system using solid lipid nanoparticles loaded with VRC VRC-SLNs) prepared by high-shear homogenization and ultrasonication methods. Large-scale productions of lipid nanoparticles are mainly obtained by high pressure homogenization technique [14]. The influence of some formulation variables on the characteristics of the VRC-SLNs was also investigated. Drug susceptibility testing was performed according to the Clinical and Laboratory Standards Institute (CLSI) M38-A2 protocol for strains both resistant and susceptible to VRC.

2. Material and method

Voricnazole (Fanavaran Daroui Hakim, Tehran-Iran), Compritol®888 ATO (CO), Lipocire, Precirol®ATO5 and stearic acid (all were supplied by Gattefossé;Saint-Priest, Cedex, France), spans 80, 60, Tween 80, sodium acetate, acetic acid, methanol, Sabouraud dextrose agar (SDA), RPMI medium (Merck, Germany) and morpholinepropanesulfonic acid (MOPS) from Sigma Chemical Co., St. Louis, MO (USA) were used. All other reagents and solvents used in the current study were either of analytical or HPLC grades.

2.1. Screening of lipids

The screening of lipids was performed by evaluating the solubility of voriconazole, VRC with varying concentrations ranging from 10 to 25% w/w with regard to lipid mass in different lipids (Compritol®888 ATO (CO), Lipocire, Precirol®ATO 5 and stearic acid). The physical mixtures of lipid and VRC were heated to 85 °C followed by the examination of the formulations to see which lipid or lipid combinations could dissolve the drug completely. Those lipids were able to dissolve the drug completely were selected to make SLNs.

2.2. Preparation of VRC-SLNs

The solid lipid nanoparticles loaded with VRC (VRC-SLNs) was prepared by high-shear homogenization followed by probe ultrasonication technique which has been published previously [15]. Large-scale productions of lipid nanoparticles are mainly obtained by high pressure homogenization technique. Briefly, the mixtures of solid lipid (2 g), VRC (0.5 g) and span 80/60 (0.25 g) were thoroughly mixed and melted at 85 °C. Then the molten mixture was transferred to the 1/3 aqueous solution containing 0.5 g of hydrophilic surfactant Tween 80 (Tn80). The mixture then was heated at the same temperature (85 °C) followed by sonication of the mixture (Bandelinsonopuls, Berlin,Germany) for 5 min to form a coarse pre-emulsion. After sonication, the obtained mixture was thoroughly dispersed in the rest of the aqueous solution containing surfactants (Tn80) cooled in an ice bath. The final mixture was maintained in the ice bath for 10 min while sonicating. This cooling step caused the formation of the lipid nanoparticles.

2.3. Characterization of VRC-SLNs

2.3.1. Determination of particle size and zeta potentials

The Zeta Sizer Nano ZS (Malvern Instruments, UK) was employed to determined the average particle size and zeta potential of SLNs at 25 °C at a fixed angle of 90°. The results are the means and standard deviations of at least three determinations.

2.3.2. Morphology measurement

In order to evaluate the morphology of drug particles field emission scanning electron microscope (FESEM, HITACHI S-4160, and U.S.A) was used. A drop of suspension was located on double-sided carbon tape and dried at 25°C for 24 h followed by sputter coated with gold for 40 s before examination.

2.4. Antifungal susceptibility testing for VRC-SLN

2.4.1. Isolates

A total of 62 clinical and environmental isolates were employed. Clinical isolates (n = 38, 61.3%) had been obtained from the lower respiratory tract, sinus, cerumen, nails. Biopsy samples via routine diagnostic procedures at hospital laboratories and environmental isolates (n = 24, 38.7%) had been collected from the soil of gardens surrounding the hospitals and indoor air of hospital wards. Cultures were prepared on a Sabouraud dextrose agar plate (SDA; Difco), and incubated at 30 °C for 72 h.