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Voriconazole into PLGA nanoparticles: Improving agglomeration and antifungal efficacy

Hai-sheng Peng [a,1], Xi-jun Liu [a,1], Gui-xiang Lv [a], Bo Sun [a], Qing-fei Kong [a], Dong-xu Zhai [a], Qi Wang [a], Wei Zhao [a], Guang-you Wang [a], Dan-dan Wang [a], Hu-lun Li [a,*], Lian-hong Jin [a], Nikolaos Kostulas [b]

[a] *Department of Neurobiology, Harbin Medical University, Heilongjiang Provincial Key Laboratory of Neurobiology, 157 Bao Jian Road, Harbin 150081, China*
[b] *Department of Neurology, Huddinge University Hospital, Stockholm S-141 86, Sweden*

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Abstract

This study is concerned with preparing PLGA nanoparticles loaded with voriconazole (PNLV), investigating the burst release and agglomeration of PNLV, and also evaluating antifungal efficacy of PNLV compared with voriconazole (VRC). The emulsion–solvent evaporation technique for nanoparticles and tests against fungi were completed. The amount of VRC in PNLV with sodium hexametaphosphate was 2.01 ± 0.27%, and burst release of PNLV was reduced by about 33% using 20% ethanol solution ($n = 3$). The mean D_{50} of PNLV with or without this salt was 132.8 nm and 6.3 μm, respectively ($n = 5$). *In vitro*; the fungal numbers treated with PNLV (3.5 mg/ml, equal amount calculated by VRC) and VRC (70 μg/ml) in tubes at the day 7 were 5.74 $\log_{10}$ and 6.72 $\log_{10}$, respectively ($P < 0.05$). *In vivo*; the fungal burden treated with PNLV and VRC in tissue from mice kidneys at day 7 after administration was 0.64 $\log_{10}$ and 2.61 $\log_{10}$, respectively (5 mg/kg, $P < 0.001$). The hematoxylin–eosin stain in mice kidney showed that the pathological lesions treated with PNLV were relieved in contrast with those with VRC. These results suggest that the emulsion–solvent evaporation process is feasible in preparing PNLV. Moreover, ethanol solution decreased burst release and Na-HMP inhibited agglomeration. PNLV could improve the VRC antifungal efficacy.

Keywords: Voriconazole; Agglomeration; PNLV; Burst release; *Candida albicans*

1. Introduction

Voriconazole (VRC) is modified from fluconazole and acquires an improving potency and spectrum (Naithani and Kumar, 2005). It can be administered via the oral and intravenous (I.V.) routes (Herbrecht, 2004). Although the oral bioavailability is estimated to be 96% (Leveque et al., 2006), it is likely that most physicians may not opt for oral therapy when initiating VRC due to the indications of the drug. However, beta cyclodextrin (BCD) derivatives containing in I.V. VRC increase the aqueous solubility of VRC but limit the application of the drug for its nephrotoxicity and haemolysis (von Mach et al., 2006).

Poly(lactide-co-glycolide) (PLGA) can be formulated into various carriers for different drug classes due to their favorable properties such as good biocompatibility, biodegradability, and safety (Jiao et al., 2002). PLGA nanoparticles loaded with voriconazole (PNLV) have all qualities of a delivery system of nanoparticles such as decreasing the fluctuation of drug concentration in blood and increasing drug bioavailability and, importantly, are free from BCD. Also, the continuous release of the drug in PNLV gradually degrades in the body fluid and avoids simultaneous breakdown of VRC. Therefore, the drawback of drug instability can be improved by the PNLV drug delivery system.

Abbreviations: BCD, beta cyclodextrin; *C. albicans*, *Candida albican*; CFU, clonal formation unit; HPLC, high pressure liquid chromatography; I.V., intravenous; Na-HMP, sodium hexametaphosphate; PBS, phosphate buffered saline; PLGA, poly(D,L-lactic-co-glycolic acid); PNLV, poly (D,L-lactic-co-glycolic acid) nanoparticles loaded with voriconazole; PVA, polyvinyl alcohol; SEM, scanning electron microscope; VRC, voriconazole.

* Corresponding author. Tel.: +86 451 8666 2943; fax: +86 451 8750 2363.
E-mail address: lihulun@yahoo.com.cn (H.-l. Li).

[1] These authors contributed equally to this work.

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The present study aims to investigate the feasibility of PLGA nanoparticles loaded with voriconazole, improve their burst release and agglomeration, and also approach the efficacy against fungi compared with voriconazole.

2. Materials and methods

2.1. Chemicals

VRC was a gift from Jiandi Pharmaceutics Technological and Development Co., Ltd. (Harbin, China). Poly(D,L-lactic-co-glycolic acid) (PLGA, Av. Mw 29,000 Data) with a lactide/glycolide ratio of 50:50, both end-capped by a suitable ester group, was synthesized and kindly provided by Shandong Research Institute of Medical Equipment (Ji'nan, China). Polyvinyl alcohol (PVA) 88% hydrolyzed (Av. Mw 30,000) was purchased from Kuraray Co., Ltd. (Korea). All other agents were of analytical grade or higher purity. Water used in the investigation was purified through pure water systems (Pall, USA).

2.2. Animals

KunMing (KM) mice purchased from the Third Affiliated Hospital of Harbin Medical University (Harbin, China) weighing 20–22 g were used in the study. They had access to food and water freely. The Institute's Animal Ethics Committee has approved all of the animal studies. The investigations conform to the *Guide for the Care and Use of Laboratory Animals* published by the US National Institute of Health (NIH Published No. 85–23, revised 1996).

2.3. Fungal inocula

Three days before the challenge, *Candida albican* (*C. albicans*) *ATCC* 10231 was subcultured daily in Sabouraud's dextrose broth. On the day of the challenge, the subculture was pelleted and rinsed twice with 0.01 M phosphate buffered saline (PBS), pH 7.2. The final pellet was resuspended in PBS. The concentration of blastospores was counted with a hemacytometer and prepared for the following experiments.

2.4. Preparation of PLGA nanoparticles loaded with voriconazole

VRC nanoparticles were prepared as previously reported by the emulsion evaporation technique (Jalil and Nixon, 1989; Jaiswal et al., 2004). Briefly, the oil phase was poured into the aqueous phase with or without sodium hexametaphosphate (Na-HMP) (pH 7.0) and dispersed by another high-speed homogenizer (IKA, T18, German) under ice cooling temperature for 1 min at 6000 rpm. The resultant emulsion was then passed through high-pressure homogenizer (Niro Soavi S.P.A., NS1001, Italy) for three times at an operating pressure of 800 bars. The nanodispersion was collected in a glass beaker and kept for 20 h on magnetic stirrer at 500 rpm (IKA, ETS-D4, German) in fume hood in order to remove CH_2CI_2. The hardened nanoparticles were centrifuged (28,300 × *g*) for 30 min and rinsed twice with pure water, freeze dried to complete the removal of organic solvent and water, and finally stored at 4 °C. The compositions of the oil and water phase in the preparation process were described in Table 1.

Table 1
The compositions of oil phase and water phase in the preparation process

Phase	Materials	Amount
Oil phase	CH_2CI_2 (ml)	5
	PLGA (mg)	250
	Span-80 (g)	1.0
	VRC (mg)	20
Water phase	Water (ml)	100
	PVA (g)	2.5
	Tween-80 (g)	0.5
	Na-HMP (%)	0 or 3

2.5. Characterization of PLGA nanoparticles loaded with voriconazole

2.5.1. Determination of voriconazole loaded into PLGA nanoparticles

5 mg of PLGA nanoparticles were dissolved in 2 ml of acetone for 30 min. The mixture was centrifuged (9167 × *g*) for 10 min. VRC concentration in the supernatant was measured at 255 nm by reverse phase HPLC using a standard curve. The HPLC was provided with Waters 600 system composed by 2487 UV detector, and Rheodyne 7725i injector (20 μl sample loop). The analytical column purchased from Phenomenex (Jupiter, C18, 5μ, 250 mm × 4.6 mm, 300 E) was eluted with mobile phase containing 42% of acetonitrile and 58% of ammonium phosphate solution (0.04 M, pH 6.0). The flow rate was 1 ml/min. Calibration curves were constructed and the volume injected into the HPLC system was 10 μl. There was a linear relationship between 0.2 and 20 μg/ml ($y = 3{,}041{,}244x - 269{,}675$, $R^2 = 0.9991$).

2.5.2. PLGA nanoparticles size distribution and morphology

The size distribution of particles was analyzed by laser particle size analyzer (Malvern, Mastersizer 2000, UK). The colloid containing PNLV was pumped into detector pool by peristaltic pump. The volumes of the particles were expressed as the mean diameter ± S.D. Data was collected from five different batches.

Freeze dried samples were dialyzed and salts were removed followed by drying onto an aluminum lamella at room temperature. The surface morphology and size of the samples were imaged by scanning electron microscope (SEM) (Hitachi, S-4700, JP).

2.5.3. Decrease of initial burst release of voriconazole from PLGA nanoparticles

VRC samples of 2 mg for the two preparations were suspended in 1 ml PBS (pH 7.4, 0.5% SDS) or 20% ethanol and violently mixed for 5 min. Then, the supernatant was removed and the VRC content was determined after centrifu-