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Development and characterization of voriconazole loaded nanoparticles for parenteral delivery

Petra Füredi, Kristóf Kovács, Krisztina Ludányi, István Antal, Imre Klebovich*

Semmelweis University, Department of Pharmaceutics, H-1092 Budapest, Högyes Endre St 7, Hungary

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ABSTRACT

Human serum albumin (HSA) has attracted the most attention in the last decades as a new nanocarrier system of active pharmaceutical ingredients (API) due to its biocompatibility and high binding capacity to hydrophobic drugs. Voriconazole (VCZ), an antifungal agent with low water solubility, was selected to produce albumin based nanoparticles using nanoparticle albumin-bound technology (nab™-technology).

Aim of our study was to study the development process of VCZ-loaded nanoparticles for parenteral drug delivery, such as homogenizing pressure, homogenizing cycle number and drug loading capacity. The main characters of nanoparticles such as particle size distribution and polydispersity index (PDI) were determined by dynamic light scattering. Six homogenization cycles at 1800 bar were ensured the acceptable PDI value (lower than 0.3) of the VCZ content nanoparticles. Optimized formulation process produced 81.2 ± 1 nm average particle size which meets the requirements of intravenous administration. Furthermore, the encapsulated concentration of VCZ was 69.7 ± 4.2% and the water solubility was over 2 times greater than the API itself which were determined by the developed HPLC method. The *in vivo* release behavior can be predicted from our applied *in vitro* dissolution study. Almost 50% of VCZ was liberated from the nanoparticles in the first 60 min.

1. Introduction

Poor solubility of drugs has always been an issue for formulation scientist thus the search for novel excipients or techniques is an ever important field in the pharmaceutical developments. Besides the conventional solubilizers (*e.g*: co-solvents, surfactants) in the past few years human serum albumin (HSA) has acclaimed a wide acceptance in drug formulation as a carrier system of active pharmaceuticals ingredient (API) (eg: Levemir®, Victoza® the albumin bound derivate of human insulin) (Elsadek and Kratz, 2012; Kratz and Elsadek, 2012). Contrarily to some conventional excipients it is biocompatible and well tolerated by the human organism without serious side-effects, such as toxicity. The binding of drugs to serum proteins is particularly important because it affects both the activity of drugs and their disposition. It has been shown that the *in vitro* binding of poorly soluble drugs to HSA during formulation can significantly increase the solubility of the drug enabling the API to be dissolved in therapeutically effective doses in an adequate aqueous dosage form to be used for parenteral administration (Kratz et al., 2007). Further research in the field proved that the use of preparations containing albumin nanoparticles does not only improve solubility of the drug but can also exhibit other advantageous effects, such as targeted therapy. Zensi A. et al. modified human serum albumin nanoparticles with apolipoprotein A-I, which can pass through the blood-brain barrier (Zensi et al., 2010). These new delivery vehicles also play an important role in oncology as most of the tumor cells secrete a special extracellular matrix glycoprotein, known as secreted protein acid and rich in cysteine (SPARC) that has high affinity to albumin. During therapy albumin nanoparticles reach the interstitium with albumin-activated gp60 pathway and they bind to the SPARC. The albumin nanoparticles-SPARC complex releases the bound drug enabling the free drug to be easily transported across the tumor cell membrane by diffusion. (Desai et al., 2006; Green et al., 2006; Sparreboom et al., 2005) Based on these finding a number of technologies were developed to prepare albumin nanoparticles: desolvation (Langer et al., 2003; Sripriyalakshmi et al., 2014; Storp et al., 2012; Weber et al., 2000), emulsification (Shen et al., 2008), thermal gelation (Boye et al., 1996; Qi et al., 2010), nano spray drying (Lee et al., 2011), nanoparticle albumin-bound technology (nab™-technology) (Desai, 2008; Desai et al., 2008; Fu et al., 2009; Thao le et al.,

* Corresponding author.
E-mail address: klebovich.imre@pharma.semmelweis-univ.hu (I. Klebovich).

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2016; Yu et al., 2015) and self-assembly techniques (Li and Yao, 2009). The characteristics of the final product of these techniques differ in particle size, size distribution and stability. For example the desolvation process of HSA nanoparticles by Weber et al. produced 300–350 nm range of particle size (Weber et al., 2000), while Shen and coworkers prepared albumin nanospheres with 200 nm particles size by emulsification technology (Shen et al., 2008). One of the most notable advantages of nanoparticle albumin technology compared to the other techniques is the fact that it can be applied in large batch production, as seen with the marketed formulation of paclitaxel (Fader and Rose, 2009; Green et al., 2006; Micha et al., 2006; Miele et al., 2009). Voriconazole (VCZ), a second-generation triazole antifungal agent, is the first line treatment of invasive aspergillosis, and has been successfully used in other invasive fungal infections, such ascandidiasis, fusariosis or scedosporidiosis. (Okabayashi et al., 2009; Pierard et al., 2003; Sanati et al., 1997; Sheehan et al., 1999) Due to its poor solubility in aqueous media (S = 0.0978 mg/ml) the marketed intravenous formulation contains sulfobutylether beta-cyclodextrin (SBEβCD) (Hafner et al., 2010). This composition is contra-indicated in patients with impaired renal function due to SBEβCD (Luke et al., 2012; Oude Lashof et al., 2012). In addition, voriconazole exhibits low stability in aqueous media because of a retro-aldol reaction (Zaludek and Zatloukalova, 2014). This phenomenon causes two issues: a, the applied cyclodextrin has different affinity to the diastereomers of voriconazole b, long-term stability. Latter is solved by freeze drying the product (Owens et al., 2000). Therefore, development of an intravenous formulation of voriconazole using nab^{TM}-technology, thus excluding cyclodextrin holds great potential. Additionally, the protein binding affinity of voriconazole is particularly high (58%) (Vanstraelen et al., 2014) which is an ideal property for the above mentioned technology. The aim of the study was to prepare and investigate the critic parameters of producing voriconazole-loaded nanoparticles (VCZ-NPs). In order to achieve the set aims our purpose was to study the effect of the applied organic solvent, concentration of VCZ, concentration of the HSA solution and ratio of the organic and aqueous solutions on the prepared nanoparticles. Furthermore, our aim was to investigate and optimize critical process parameters, such as homogenizing pressure, homogenizing cycle number and the method of organic solvent removal. Finally, our aim was to apply the optimized technology and characterize a VCZ-NP by particle size, particle size distribution, loading capacity and *in vitro* dissolution.

2. Materials and methods

2.1. Materials and reagents

Voriconazole was purchased from BrightGene Bio-Medical Technology Co., Ltd., China, lyophilized human serum albumin (96–99%) from Sigma-Aldrich, Hungary and 20% infusion of human serum albumin from Octapharma, UK. Phosphate buffered saline (PBS), chloroform, isooctanol and the acetonitrile were purchased from Sigma-Aldrich, Hungary.

2.2. Preparation of VCZ-NPs

VCZ-NPs were prepared by nab^{TM} technology. During the determination of the homogenization parameters various organic solvents (isooctane, chloroform) and various ratios of organic solvent and aqueous solution of HSA were applied. Firstly 10 mg voriconazole was dissolved in 1 ml organic solvent. Human serum albumin infusion was diluted with Milli-Q water to 2 or 3%. Following the mixing of HSA solution with voriconazole solution the mixture was prehomogenized for 3 min at 80 rpm using a Homorex mixer (Brogli& Co. AG., Switzerland). The prehomogenized mixture was then further homogenized to form VCZ-NPs using an Avestin Emulsiflex B15 (Avestin, Germany). The resulting colloidal system was lyophilized (ScanVac Coolsafe TM) for 24 h at +5 °C shelf temperature.

2.3. Characterization of VCZ-NPs

2.3.1. Particle size measurement

The lyophylized nanoparticles were dissolved in 2.5 ml of water and filtrated through 0.2 μm microporous syringe filters to remove any undissolved particles. Samples were then diluted ten-fold with distilled water. The parameters of the particle size measurement were: material: protein, refractive index: 1.450, temperature: 25 °C, equilibration time: 120 s, cell type: PCS115 glass cuvette.

2.3.2. Differential scanning calorimetry (DSC)

Accurately weighted samples (2 mg) were placed in non hermetically sealed aluminum pans and heated at a rate of 10 °C/min to a maximum of 270 °C using a Seiko Exstar 6000 DSC. An empty aluminum pan was used as a reference.

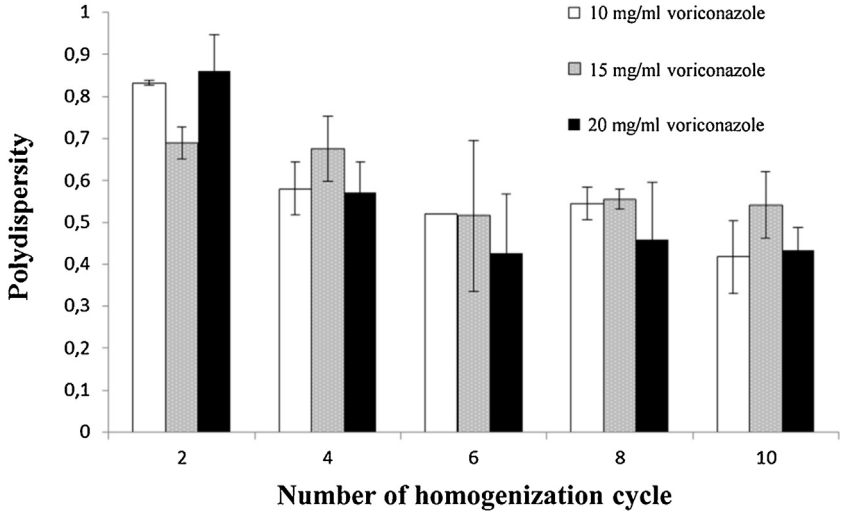

Fig. 1. Influence of the number of homogenization cycle on the polydispersity.