Technical Report:

The Antimicrobial Activity of Itraconazole and LoxaSperse® Against Biofilms of *C. albicans*

Abstract: Itraconazole is a broad-spectrum, triazole antifungal agent, class II drug molecule (low solubility–high permeability) according to the Biopharmaceutical Classification System (BCS). LoxaSperse is an excipient manufactured by PCCA and can be used as a chemical dispersing or solubilizing agent in irrigation or nebulization formulations, improving the solubility and dispersibility of poorly water soluble Active Pharmaceutical Ingredients (APIs). The *in vitro* antimicrobial activity of itraconazole in a LoxaSperse formulation was evaluated against *Candida albicans* biofilms and compared to the same activity of reference antifungal drugs (itraconazole, fluconazole and amphotericin B), in order to verify the benefits of the LoxaSperse formulation. The LoxaSperse formulation reduced Minimum Biofilm Inhibitory Concentration (MBIC) 10-fold compared to the value of itraconazole alone. Improvement in antimicrobial activity of the LoxaSperse/itraconazole formulation could be attributed to the improved dissolution rate and solubility enhancement caused by the base over the poorly water-soluble itraconazole.

Purpose:

To evaluate the *in vitro* antimicrobial activity of itraconazole in a LoxaSperse formulation, Loxasperse alone, and Itraconazole EP Micronized, fluconazole and amphotericin B (reference antifungal drugs) against *Candida albicans* biofilms.

Introduction:

Local delivery of medication to the sinuses and lungs is highly desirable, especially in patients with specific sinus and pulmonary diseases such as cystic fibrosis, asthma, chronic sinus and pulmonary infections, and lung cancer. The principal advantages include reduced systemic side effects and higher doses of the applicable medication at the site of drug action (Harvey and Schlosser, 2009; Pilcer and Amighi, 2010).

Many existing APIs and an increasing number of new drugs are often poorly water-soluble drugs (Zhang *et al.*, 2011). Drug insolubility, regardless of the administration route, commonly generates bioavailability or efficacy problems. Different techniques exist to increase drug dissolution and/or solubility, which often require the use of specific excipients. In the ear, nose and throat (ENT) injuries and illness field, excipients should be chemically and physically stable, inert to the API and exhibit no side effects (Duret *et al*., 2012).

LoxaSperse is a proprietary excipient manufactured by PCCA for use as a chemical dispersing or solubilizing agent in oral, sinus, inhalation, rectal and topical formulations. It consists of a blend of micronized xylitol and micronized poloxamers, designed to be mixed with APIs in order to improve their water solubility and dispersability (PCCA, 2013). Xylitol is a 5-carbon sugar with low transepithelial permeability which is poorly metabolized by bacteria (Durairaj *et al*., 2007). Poloxamers are a series of synthetic block copolymers of poly(ethylene oxide-b-propylene oxide-b-ethylene oxide) (PEO–PPO–PEO) with varying molecular weights and block ratios. They are non-ionic amphiphilic surfactants possessing excellent wetting, antifoaming and solubilizing properties (Moebus *et al*., 2009). The use of xylitol and poloxamers in nebulization and irrigation is thoroughly referenced in the literature and there is evidence of their safety. (Durairaj *et al*., 2007; Jagannath *et al*., 1995; Plataki *et al*., 2011; Zabner *et al*., 2000). LoxaSperse is a base that allows for the preparation of non-sterile capsules and powder sachets that are added to sterile water or normal saline by the patient at the moment of administration (PCCA, 2013).

Candida infections have increased dramatically over the past years, being reported as the fourth most common nosocomial bloodstream pathogen. Candidemia represents 10% of all nosocomial blood-stream infections (Burgess *et al*., 2000).
The traditional treatment uses amphotericin B, but it has changed to relatively less toxic alternatives, such as the triazole antifungals itraconazole and fluconazole (Wroblewska *et al*., 2002).

Itraconazole has a broader spectrum of activity than other azole antifungals (De Beule, 1996). However, poor oral bioavailability, variable absorption and gastrointestinal toxicity due to the hydroxypropyl-β-cyclodextrin component of the oral solution limit itraconazole to a second or third line treatment option for invasive fungal infections (Vaughn *et al*., 2007). Itraconazole is a typical Biopharmaceutical Classification System (BCS) Class II drug with low solubility–high permeability (Amidon *et al*., 1995). An inhaled itraconazole delivery system has shown an interesting potential for treating pulmonary invasive fungal infections with improvement of its efficacy (Duret *et al*., 2012).

Methodology:

Materials: Itraconazole EP Micronized (lot number C149307) and PCCA Formula #10342 (4 g of Itraconazole EP Micronized + 37.574 g of LoxaSperse) were provided by PCCA (Houston, TX, USA) as powders. Itraconazole and PCCA Formula #10342 were prepared on the day of the assay. Fluconazole and amphotericin B (Sigma Aldrich®) were obtained as powders and stored at 4°C. Stock solutions (10.24 mg/mL) of these two reference actives were prepared in sterile water.

Strain: *Candida albicans* isolate ATCC 90028 was obtained from American Type Culture Collection (Manassas, VA) and used in the course of this study.

Methods: A Minimum Biofilm Inhibitory Concentration (MBIC) of itraconazole in a LoxaSperse formulation, LoxaSperse excipient, itraconazole, fluconazole and amphotericin B was measured for the *C. albicans* biofilm according to the NCCLS M27-A broth microdilution method (NCCLS, 1997). The testing medium used for growing was RPMI 1640 (American Biorganics, Inc., Niagara Falls, NY) supplemented with L-glutamine (Sigma Aldrich®). Yeast inocula (100 µL of 1 x 10^6 cells/mL) were added to each well of 96-well microtiter plates (Corning) and incubated at 37°C for 48h. After biofilm formation, medium was aspirated and non-adherent cells were

removed by thoroughly washing the biofilms three times in sterile phosphate-buffered saline (PBS, Sigma Aldrich®). The antifungal drug and LoxaSperse solutions (samples) were then added to the biofilms in serially diluted concentrations (1,024 to 0.5 µg/ml, from stock [concentrated] solutions of each sample prepared in RPMI medium directly) and incubated for a further 48h at 35°C. A series of sample-free wells and biofilm-free wells were also included to serve as positive and negative controls, respectively. The MBIC was defined as the lowest concentration of sample that produced a 50% reduction of fungal growth compared with the growth control. Cell viability was determined by using CellTiter 96® Non-Radioactive Cell Proliferation Assay (Promega, 2013).

Results and Discussion:

All biofilms formed on the microtiter plates over 48h displayed consistent CellTiter 96® dye solution readings when the intensity of the colorimetric product was measured in a microtiter plate reader at 570 nm. The MBIC value of itraconazole in a LoxaSperse formulation (expressed as concentration of itraconazole) showed efficient result in comparison with the MBIC values for raw itraconazole, fluconazole and amphotericin B tested against *C. albicans* ATCC 90028, as reported in Table 1. The LoxaSperse formulation improved the antimicrobial potential of itraconazole approximately 10-fold. Biofilm from *C. albicans* strain tested was intrinsically resistant to fluconazole (MBIC > 1024 µg/mL). The polyene antifungal amphotericin B was highly active (MBIC = 0.5 µg/mL) against *C. albicans* ATCC 90028. The findings for fluconazole and amphotericin B are in accordance with the literature (Ramage *et al*., 2001).

Table 1. *Minimum Biofilm Inhibitory Concentrations against C. albicans ATCC 90028.*

Sample	Minimum Biofilm Inhibitory Concentration (MBIC) (ug/mL)
Amphotericin B	0.5
Fluconazole	>1,024
Itraconazole	1024
LoxaSperse	>10,240
Itraconazole/LoxaSperse	98.5

Conclusions:

Itraconazole has an increased *in vitro* antimicrobial activity against *Candida* biofilms when associated with the LoxaSperse excipient. It may be due to the benefits caused by the base in terms of the dissolution rate and saturation solubility of the poorly water-soluble itraconazole, providing a higher *in vitro* dissolved drug concentration that induced an enhanced inhibition of microbial growth.

Financial Disclosure:

For this study, PCCA contracted a third party laboratory with no proprietary or financial interests in the test products, or equity interest in PCCA.

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