Antifungal assay for an ophthalmic formulation of amphotericin B-microemulsion

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INTRODUCTION AND OBJECTIVES

Ocular fungal infections are an important ophthalmologic problem causing significant ocular morbidity (Carrasco 2011). The pharmacological approach of management of these infections involves administration of antifungals agents, such as amphotericin B (AmB) in its micellar form, Fungizon®. However the owing physiologic constraints of the eye and the low concentration of this drug achieved by the optical route define insufficient bioavailability (Gratieri 2010, Mazouri 2001, Thomas 2003).

Microemulsion (ME) is a system that contains water and oil coexisting in thermodynamic equillibrium due to the presence of a surfactant film at the oil-in-water interface. They are clear, stable, transparent and isotropic systems and currently have aroused the interest of pharmaceutical scientists because of their capacity to be used for ocular applications (Pestana 2008, Vandamme 2002). A microemulsion containing amphotericin B (AmB-ME) was proposed to fulfill this need.

The aim of this work was to perform antifungal susceptibility tests with this system against *Candida* strains in order to evaluate the minimum inhibitory concentration (MIC) and the drug profile when incorporated into the system.

MATERIALS AND METHODS

The ME formulation, phosphate buffer pH 7.4 solution and Lipoid® S100 (as water phase) and Miglyol®812N and Tween®80 (as oil phase), was prepared from a pseudo-ternary phase diagram procedure (Vandamme 2002). Both water and oil phases were magnetically stirred until complete homogenization. The ME was achieved by addition of the water phase to the oil phase followed by sonication process and ultrasound bath.

The drug was incorporated into the system at a concentration of 3.0 mg/mL. Briefly, AmB was dissolved (30 mg/mL) in NaOH solution (1 N) and added to the ME, under magnetic stirring. The pH was adjusted using hydrochloric acid (HCl) solution (1 N) to yield a 7.5-8.0 range.

The MIC was determined by the agar dilution method proposed by the National Committee for Clinical Laboratory Standards (NCCLS) against clinical isolates of *Candida* (M27-A2). These tests were performed using the AmB-ME, standard AmB as positive control and blank ME as negative control. Ninety six well plates, previously seeded with *Candida albicans*, *Candida parapsilosis* and *Candida tropicalis*, were incubated at 35ºC for 24 h and the MICs were defined as the lowest AmB concentration at which there was complete absence of growth (NCCLS 2008).

RESULTS AND DISCUSSION

The AmB-ME system used in this study (Table 1) presents favorable characteristics to ocular drug delivery. It has a homogeneous aspect, transparent appearance and absence of precipitates which suggests a typical Winsor IV system (Winsor 1948). The AmB was incorporated to this system suggesting that the drug was partitioned into the oil and aqueous phase favored by the alkaline environment, which promotes the better performance in terms of content in these structures (Qing-Ping 2009) (Figure 1).

Table 1. Composition of the ME system

Components	ME ($\%_{w/w}$)
Miglyol® 812 N	11.0
Lipoid® S100	6.3
Tween® 80	14.7
Phosphate buffer pH 7.4 solution	68.0

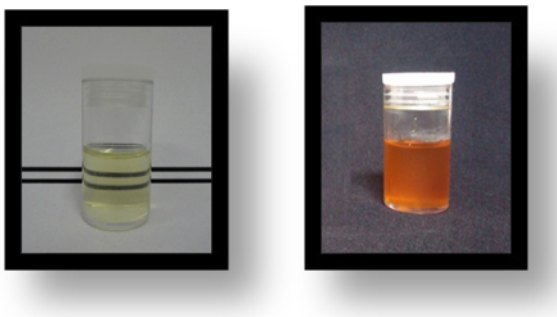

Figure 1. ME and AmB-ME picture

The need for antifungal susceptibility testing has been steadily growing over the last two decades specially driven to the emergence of antifungal resistance. Susceptibility tests are important for choosing the most appropriate treatment for patients with fungal infections (Vale-Silva 2006). Besides, these tests may contribute to evaluate the performance of new drug

delivery systems, such as ME as carrier to antifungal agents.

Table 2 summarizes the MIC values of AmB-ME susceptibility. In this experiment, the blank ME did not provide growth. This can be explained by the fact that ME can involve the lipid layer due to its unique feature that enhance the intrinsic permeation of membranes (Al-Adhan 2000). The MICs determined by the standard AmB were in agreement with established range recommended by the NCCLS document (NCCLS 2008). The *Candida* strains were considered sensitive to incorporated AmB, in which the MIC values were lower than 1 µg/mL.

Table 2: Antifungal susceptibility for AmB-ME against *Candida* strains

Fungal Strains	Range	MIC (µg/mL)
Candida albicans	0.062-0.125	0.062
Candida parapsilosis	0.250-0.500	0.375
Candida tropicalis	0.250-0.500	0.250

CONCLUSION

The results suggests that the ME system containing Lipoid® S100/Tween® 80 as surfactants, Mygliol® 812N, as oil phase and phosphate buffer, as aqueous phase, in the studied proportions, seems to be a valuable delivery system in terms of easy manufacturing and high compatibility with the requirements of the ocular route.

The ME system was able to carry the AmB and the data showed that the incorporation method was important to help on the entrapment process of this drug into this lipidic structure. Therefore, it can be considered a promising system to control *Candida* ocular infections.

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