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Amphotericin B-Loaded Poly(lactic-co-glycolic acid) Nanofibers: An Alternative Therapy Scheme for Local Treatment of Vulvovaginal Candidiasis

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ABSTRACT

Vulvovaginal candidiasis is an inflammation localized in the vulvovaginal area. It is mostly caused by *Candida albicans*. Its treatment is based on the systemic and local administration of antifungal drugs. However, this conventional therapy can fail owing to the resistance of the *Candida* species and noncompliance of patients. Amphotericin B-loaded poly(lactic-co-glycolic acid) nanofibers are single-use, antifungal, controlled drug delivery systems, and represent an alternative therapeutic scheme for the local treatment of vulvovaginal candidiasis. Nanofibers were characterized by analytical techniques and with an *in vitro* drug delivery study. *In vitro* and *in vivo* fungicidal activity of amphotericin B released from nanofibers was evaluated using the agar diffusion method and an experimental murine model of vulvovaginal candidiasis, respectively. Analytical techniques showed that amphotericin B was physically mixed in the polymeric nanofibers. Nanofibers controlled the delivery of therapeutic doses of amphotericin B for eight consecutive days, providing effective *in vitro* antifungal activity and eliminated the *in vivo* vaginal fungal burden after 3 days of treatment and with only one local application. Amphotericin B-loaded poly(lactic-co-glycolic acid) nanofibers could be potentially applied as an alternative strategy for the local treatment of vulvovaginal candidiasis without inducing fungal resistance, yet ensuring patient compliance.

Introduction

Vulvovaginal candidiasis is an inflammation localized at the vulva and the vagina. Vulvovaginal candidiasis is caused by *Candida albicans* in 85%-90% of the diagnosed cases. However, a nonalbicans *Candida* species may also be involved in vaginal yeast infections.[1] *Candida glabrata*, *Candida tropicalis*, *Candida krusei*, and *Candida parapsilosis* are the most common nonalbican species that cause vulvovaginitis.[2]

Vulvovaginal candidiasis caused by *Candida albicans* and other nonalbican *Candida* species occurs in immunosuppressed and healthy women, particularly in those with subnormal T-lymphocyte responses to Candida,[3] acquired immunodeficiency syndromes, and in transplanted patients. In addition, women affected by diabetes mellitus, who use contraceptives, and frequent antibiotics, are also susceptible to the colonization by *Candida* species.[4] The *Candida* species are opportunistic, highly virulent microorganisms. The mechanism of virulence includes their capabilities to defend against the host immune system, hyphae proliferation, biofilm formation,

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and production of hydrolytic enzymes, such as proteases, phospholipases, and hemolysin.[5]

A variety of antifungal drugs is available to treat the acute vulvovaginal candidiasis, including polyenes (nystatin and amphotericin B), imidazoles (clotrimazole, miconazole nitrate, econazole nitrate, fenticonazole nitrate), triazoles (fluconazole and itraconazole), or ciclopirox olamine.[6-8] These drugs can be systemically or topically administered, and the mycological and clinical success of the therapy range from 85% at 1-2 weeks and 75% at 4-6 weeks after treatment.[8] Recurrence of vulvovaginitis can occur shortly after the end of the treatment of acute vulvovaginal candidiasis, and in that instance, the infection can become a chronic disease.[9] The treatment of chronic vulvovaginal candidiasis involves the use of the same antifungal drugs as those we referred to earlier. However, the therapeutic approach represents a challenge because it requires a prolonged regime of suppression of the fungus and maintenance of the nonpathological condition. For example, triazoles (fluconazole or itraconazole) should be administered for periods spanning months to a year to guarantee the remission of the disease.[7]

In addition to the necessity of a prolonged treatment, the repetitive exposition of *Candida albicans* strains as well as the other nonalbicans *Candida* species leads to the occurrence of antifungal resistance. Coste et al.[10] reported the increased azole resistance of *Candida albicans* strains after a prolonged administration of fluconazole. Sobel[7] reported that *Candida glabrata* and *Candida krusei,* isolated from vulvovaginal infections, showed reduced sensitivity to fluconazole. Moreover, the extended exposition to antifungal drugs can also induce drug interactions and side effects, which are limiting to some patients.[11] Therefore, all these combined factors can lead to noncompliance to therapy, thereby resulting in an unsuccessful treatment.

To overcome the highlighted problems involved in the treatment of vulvovaginal candidiasis, the development of novel pharmaceutical dosage forms is required, such as intravaginal drug delivery systems. These nonconventional formulations can be inserted directly at the infectious focus providing a controlled and prolonged release of antifungal drugs after a single administration. As the frequency of administration is reduced and the systemic circulation of drugs is avoided, the possibility of manifesting any of the side effects as well as drug interactions can be reduced/eliminated.[12] As a consequence, the satisfaction and compliance of the patients can be profoundly increased.

In this context, the objective of this study was the development of amphotericin B-loaded poly(lactic-co-glycolic acid) (PLGA) nanofibers as alternative drug delivery systems to locally treat the vulvovaginal candidiasis. Amphotericin B is a polyene that is highly effective against *Candida albicans* and nonalbicans *Candida* species. This drug is capable of binding to the ergosterol of the fungal cell membrane, thereby forming transmembrane pores. Consequently, the membrane is depolarized and its permeability to monovalent protons and cations is increased concurrently with the flux of intracellular molecules to the external environment. All these events result in an osmotic imbalance and loss of essential molecules, inducing cell death.[13] Nanofibers are nanostructured biomaterials composed of interconnected polymeric fibers. Nanofibers exhibit distinctive and appealing characteristics compared to the bulk material owing to their small dimensions and large surface-to-volume ratios.[14,15] In addition, nanofibers are porous, which leads to the diffusion of drugs and bioactive molecules previously incorporated into the polymeric matrix in a controlled manner.[16] Considering the effectiveness of amphotericin B and the possibility of its incorporation into electrospun PLGA nanofibers, novel nanostructured drug delivery systems were designed, characterized by the use of different analytical techniques and evaluated as a therapeutic alternative to eliminate the *Candida albicans in vitro* and in a murine model of vulvovaginal candidiasis. It was hypothesized that intravaginal amphotericin B-loaded PLGA nanofibers can release this antifungal drug in therapeutic doses for a prolonged period, inducing the suppression of candidiasis in the vulvovaginal region. As a consequence, fungal resistance, systemic adverse effects and drug interactions, and patient noncompliance, can be eliminated.

Materials and Methods

Preparation of Amphotericin B-Loaded PLGA Nanofibers

A poly(lactic-co-glycolic acid) (PLGA, 75:25, Mw = 76,000-115,000 g mol^{-1}, iv. 0.71-1.0 dL/g, 0.1%[w/v] in chloroform at 25°C; Sigma-Aldrich) (61 mg) and amphotericin B (Unianf—União Química, Brazil) (3 mg) solution was prepared by dissolving PLGA and amphotericin B in 2,2,2-trifluoroethanol (purity >99%; Sigma-Aldrich) using magnetic stirring for 4 h at room temperature. This solution was transferred to a glass syringe (volume of 5 mL) with a metal needle and adapted to the electrospinning device. To produce amphotericin B-loaded PLGA nanofibers, a voltage of +25 kV was applied at a distance of 10 cm between the needle tip and collector. Nanofibers were collected on coverslips maintained over the metallic collector disc (diameter = 8 cm) at a flow rate of 3.6 mL/h. The temperature and relative humidity on the electrospinning chamber were approximately 27°C and 65%, respectively. Unloaded PLGA nanofibers were also produced by the electrospinning technique.

Characterization

Scanning electron microscopy (SEM) was performed by using an FEI microscope, INSPECT S50, and a metallizer (SPI Supplies—sputter coater). Amphotericin B-loaded PLGA nanofibers and PLGA nanofibers were mounted on aluminum stubs and coated with a gold layer. Nanofiber surfaces were analyzed at magnifications of 1000-10,000×. Adobe Photoshop 6.0 (Adobe Systems Incorporated) was used to adjust the photomicrographs.

Infrared spectrophotometry was performed using a Fourier transform infrared spectrophotometer (FTIR; Nicolet 6700 Thermo Scientific) and an attenuated total reflectance technique. Spectra of amphotericin B-loaded PLGA nanofibers, PLGA nanofibers, PLGA, and amphotericin B were obtained after 32 scans at a resolution of 1 cm^{-1}.

Differential scanning calorimetry (DSC) was performed using the EXSTAR DSC 7020 apparatus. The calorimeter was calibrated by using pure indium melting (melting point 156.6°C and $\Delta H = 25.45$ J g^{-1}). Amphotericin B-loaded PLGA nanofibers and PLGA nanofibers were put into aluminum pans. They were heated from 20°C to 180°C at a heating rate of 20°C min^{-1} in the presence of nitrogen. The software OriginPro 8.0 was used to plot the thermograms.

Thermogravimetry was performed using the EXSTAR DSC 7020 apparatus. Amphotericin B-loaded PLGA nanofibers, PLGA nanofibers, PLGA, and amphotericin B, were placed into aluminum pans. They were heated from 25°C to 700°C at a heating rate of 10°C min^{-1} in the presence of nitrogen. OriginPro 8.0 was used to plot the thermograms.

Content Uniformity of Amphotericin B Incorporated Into PLGA Nanofibers

Conducted work complied with the general chapter of the United States Pharmacopoeia.[17] Ten nanofibers (diameter = 6 mm) were weighted, and the amphotericin B in each nanofiber was