Preparation and Characterization of Mucoadhesive Thermoresponsive Systems Containing Propolis for the Treatment of Vulvovaginal Candidiasis

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ABSTRACT: This work describes the preparation and characterization of mucoadhesive thermoresponsive systems consisted of poloxamer 407 (P407), Carbopol® 934P (C934P), and propolis to treat vulvovaginal candidiasis (VVC). Systems were obtained with different percentages of P407 and C934P to deliver propolis, a potent drug against VVC. Temperature of gelation, hardness, compressibility, adhesiveness, elasticity, cohesiveness, mucoadhesion, rheology (continuous flow and oscillatory), *in vitro* drug release, and antimicrobial activity were evaluated. Increasing the polymer content or temperature and the drug presence significantly increased mechanical properties of formulations. These exhibited pseudoplastic flow and low degrees of thixotropy. In most samples, increasing the C934P content significantly changed the oscillatory rheological properties. Formulations showed thermoresponsive behavior, existing as a liquid at room temperature and gel at 34°C–37°C. Propolis release from formulations was controlled by phenomenon of relaxation of polymer chains or displayed anomalous behavior, dependent of concentration of each polymer. The *in vitro* antimicrobial activity of preparations was evaluated against microorganisms of vaginal importance (*Candida albicans*, *Candida glabrata*, *Candida parapsilosis*, *Candida tropicalis*, and *Saccharomyces cerevisiae*), displaying activity against all yeast tested. The data obtained for these systems indicate a potentially useful role in the treatment of VVC and suggest they are worthy of clinical evaluation. © 2013 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 102:1222–1234, 2013

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INTRODUCTION

Vulvovaginal candidiasis (VVC) is an infection of the female genital tract caused by the abnormal growth of yeast-like fungi, being classified by the World Health Organization as a sexually transmitted disease of frequent sexual transmission.[1–3] Opportunistic pathogens are often isolated from the mucosal surfaces of normal individuals,[4–6] being associated with asymptomatic (ASS) colonization or as filamentous forms (pseudohyphae and true hyphae) observed in pathogenic processes.[5] Moreover, VVC is caused mainly by the genus *Candida*,[5,7] where 80%–90% of cases are due to *Candida albicans* and 10%–20% are due to the other species called not-*C. albicans* (*Candida tropicalis*, *Candida glabrata*, *Candida krusei*, *Candida parapsilosis*, *Candida pseudotropicalis*, and *Candida lusitaniae*).[6,8,9] *C. glabrata* is the second species in frequency in the VVC.[5,6,10] However, yeast of other genus may also cause this infection, such as *Saccharomyces cerevisiae*, *Rhodotorula* sp., and *Trichosporon* sp.[5] Approximately 5% of women with VVC develop recurrent VVC (RVVC), usually defined as the occurrence of four or more episodes of VVC in the period of 12 months.[5,6] Moreover, studies indicate that 20%–25% of women usually healthy

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are completely ASS.[5,10] The manifestations of VVC are often painful and uncomfortable (intense pruritus vulvae, leukorrhea, dyspareunia, dysuria, edema, and vulvovaginal erythema).[5,6] Thus, by affect, millions of women annually, determining great discomfort, interfering in sexual and affective relations, and damaging the job performance, VVC is considered an important global public health problem.[6,13]

The treatment of this disorder has not obtained good results. In the most cases, the relief of symptoms is temporary and the average rate of cure is 75%–80%, with constant relapses. About 30 years ago, the introduction of imidazole derivatives, such as ketoconazole, clotrimazole, miconazole, tioconazole, and later, itraconazole and fluconazole (FLU), made it possible to increase this rate to 85%–90%. However, they are no antifungal agents for specific use in VVC.[6] In addition, hepatotoxicity, gastrointestinal disorders, and the high cost are other limiting factors for the use of these drugs, and they make the use of them "prohibitive," especially in the public health system.[6,14] Furthermore, none have been able to eradicate the symptoms once, and the problem of relapse remains.[6,15] Moreover, the treatments could cause the eradication not only of pathogenic microorganisms, but also the restoration of normal vaginal microbiota.[16] However, evidence suggests that the development of tolerance, occurrence of side effects, and the increase at the rate of infection recurrence are mainly due to a failure on taking place the normal vaginal microbiota, composed of *Lactobacillus* after antimicrobial therapy.

In this sense, propolis showed to be effective in the treatment of vaginal infections and is not associated with the formation of microbiological resistance.[6,16,17] It is a bud-resin strong adhesive produced by bees *Apis mellifera* L. that is already being used by men since ancient times for its pharmaceutical properties, including antimicrobial,[6,18–20] anti-inflammatory,[21,22] and antioxidant activity.[23,24] Moreover, propolis is relatively nontoxic and because of its safety and efficacy, it has been used in release systems to treat CVV, mainly in the form of microparticles.[6,25–28]

Additionally, the inability of traditional local dosage forms to get an intimacy contact with the vaginal mucosa during an ideal residence time and the potential side effects of administering systemic antibiotics have fueled interest in the sustained delivery of therapeutic agents on the mucosa. Consequently, mucoadhesive systems designed to release drugs from several polymeric systems, together with their rheological, mechanical, and clinical evaluation, have been reported.[29] In this sense, pharmaceutical polymer mucoadhesive systems enable to control the location and the release of the drug in a particular region of the body, increasing the availability and the contact time between this and the mucosa.[28,30,31] In the vaginal mucosa environment, the polymers interact with the mucin-coated epithelial by means of specific interfacial forces in a process commonly referred as mucoadhesion, a special case of bioadhesion.[28–30,32] Furthermore, Carbopol 934P® (C934P) and poloxamer 407 (P407) have been employed as blends in the development of mucoadhesive thermoresponsive systems because of its physical and chemical characteristics and interaction with the mucosa.[27,28,33–35]

Therefore, the present work describes the development and characterization of mucoadhesive thermoresponsive systems containing propolis prepared from P407 and C934, designed as a platform for vaginal application for the treatment of VVC.

MATERIALS AND METHODS

Materials

P407 and C934P were purchased from Sigma (St. Louis, Missouri) and from B.F. Goodrich (Brecksville, Ohio), respectively. Triethanolamine (TEA), purchased from Galena (Campinas, SP, Brasil), was used as a neutralizing agent. Chloramphenicol was purchased from União Química (Embu-Guaçu, SP, Brazil), nystatin (NIS) from Sigma, and FLU from Pfizer (São Paulo, Brazil). Furthermore, Roswell Park Memorial Institute-1640 culture medium (RPMI) was purchased from Gibco (Chicago, Illinois), and *Sabouraud* Dextrose Agar (SDA) medium was purchased from HiMedia Laboratories (Mumbai, India). All other chemicals were purchased from Merck (Darmstadt, Germany) and were of AnalaR, or equivalent, quality.

Preparation of Propolis Extractive Solution

Brazilian propolis sample was collected from hives of *Apis mellifera* L. bees at a farm of Cianorte City, Paraná State, Brazil. The apiary was located northwest of Paraná State, inside a eucalyptus reserve surrounded by native forest with a predominance of *Baccharis dracunculifolia* (Asteraceae). The sample were triturated and maintained at the temperature of −20°C until further analysis. Propolis extractive solution (PES) was prepared with a propolis/ethanol ratio of 30/70 (w/w) by turbo extraction, at 3500 rpm for three times at 15 min with two intervals of 5 min. PES was filtered through filter paper, and made up to the initial weight with the ethanol.[25,28]

Preparation of Mucoadhesive Thermoresponsive Formulations

The required mass of C934P (0.10%, 0.15%, 0.20%, or 0.25%, w/w) was dispersed in distilled water using a mechanical stirrer. Following complete dissolution, P407 (15% or 20%, w/w) was added to this dispersion and the mixture was stored at 4°C for 12 h to ensure