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Improved vaginal retention and enhanced antifungal activity of miconazole microsponges gel: Formulation development and in vivo therapeutic efficacy in rats

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ABSTRACT

Traditional azole antifungal formulations suffer from poor retention in the vaginal cavity, irritation and burning of the vaginal area. In the present work, we aim at the development of a novel miconazole (MCZ) microsponges gel as an attractive dosage form for vaginal candidiasis. The proposed formula has the potential to minimize the local side effects of the drug due to the controlled release characteristic, which increases patient compliance. Moreover, the mucosal retention effect of the microsponges in addition to the bioadhesion property of Carbopol gel prolongs the retention of the dosage form in the vagina and consequently improves the therapeutic efficiency. MCZ microsponges were prepared applying Quasi emulsion method using Eudragit RS100. The effect of formulation factors, namely, drug:polymer ratio (1:1, 2:1 and 4:1), the amount of poly vinyl alcohol (PVA) (25, 50 and 75 mg) and the volume of organic solvent (2.5, 5, 10 mL) on the characteristics of MCZ microsponges has been investigated. The microsponges were optimized regarding the production yield (68.8 ± 6.4%), particle size (78.2 ± 2.1 μm), entrapment efficiency (92.9 ± 1.9%) and release rate (Q150 51.8 ± 2.5%). The selected formula was further evaluated for its, flowability, porosity and surface morphology. MCZ microsponges were incorporated into Carbopol gel, then the viscosity and bioadhesion were examined. The in vitro antifungal activity of MCZ microsponges gel was comparable to the market product. In vivo, MCZ microsponges vaginal gel was more effective than the market product ($p < 0.05$) in eradicating *Candida* infection in rats, which was supported by the histopathological findings.

1. Introduction

Up to 80% of women experience at least one episode of vaginal candidiasis in their life. *Candida albicans* is the most common cause of the condition, responsible for almost 85% of the infections (http://www.cdc.gov/fungal/diseases/candidiasis/genital/). Many effective therapies are available to treat this condition, but there are still a number of limitations for each of them. Systemic side effects and the possibility of interactions with other medications are the main obstacles for the oral dosage forms. Although considered safe, available vaginal preparations suffer from the disadvantage of low retention in the vaginal epithelium. In addition, they have been undesirable for women due to compliance problems; messy, dripping creams and night-time dosing (Merabet et al., 2005). In order to overcome the weaknesses of traditional vaginal delivery systems without influencing the efficacy of the treatment, new vaginal formulations are in demand.

Miconazole (MCZ) (1-2-((2, 4 dichlorophenyl)-2-(2, 4-dichlorophenyl)-methoxy) ethyl)-1-imidazole) is an azole antifungal, which has been widely used in the local treatment of yeasts and dermatophytes infections (Sawyer et al., 1975). The solubility of miconazole in water is 0.763 mg/L, its logP value is 5.86 and its pKa is 6.77 (https://pubchem.ncbi.nlm.nih.gov/compound/miconazole#section = WIPO-IPC, 2017). Miconazole has been proved equally effective in both candida and dermatophyte infections of the vagina, skin and oral mucosal. Miconazole inhibits steroidogenesis, interrupts steroidogenic acute regulatory protein expression, inhibits Ca^{2+}-activated K^{+} channel and suppresses agonist-induced protein-tyrosine phosphorylation (Chang et al., 2005). Miconazole is among the antifungal agents proven to be effective in vulvovaginal candidiasis. Miconazole is available as 2% vaginal cream, 100-mg suppositories to be inserted in the vagina at bedtime for 7 days, and 200-mg vaginal suppositories for 3-day treatment. Miconazole may cause side effects such as increased burning, itching, or irritation of the vagina, fever and foul-smelling vaginal discharge. Reinfection is the most common drawback in MCZ

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vaginal formulations, because patients often stop using the treatment before complete eradication of the fungus (Barnhart, 2005). Therefore, controlled-release delivery system would minimize the side effects of MCZ and provide a long-term therapeutic concentration would be clinically significant (Park, 2014).

Microsponges consist of porous microspheres that encapsulate a variety of materials like medications, essential oils, sunscreens, fragrances and emollients. Microsponge matrix consists of a huge number of interconnecting pores in a non-collapsible structure. When applied to the mucous membranes, microsponges get retained in the small cavities and folds and slowly release the encapsulated material. This adds a safety advantage to the microsponges because they don't reach the systemic circulation. Furthermore, due to their small pore diameter, bacterial cells with a size of 0.007 to 0.2 μm cannot infiltrate into the voids of microsponges. According to the use and the route of administration, the size of microsponges ranges from 5 to 300 μm. It was found that a 25 μm microsponge particle can have up to 250,000 pores and total pore volume of approximately 1 mL/g, which provides a large space for drug loading (Embil and Nacht, 1996). The controlled release pattern of microsponges prevents the accumulation of excess amount of the drug at the application site which decreases the potential of skin irritation. Due to the above mentioned advantages, microsponges are commercially available in cosmetics, over-the-counter skin care products, sunscreens, anti-inflammatory agents in various forms including lotions, creams and gels. In this context, benzoyl peroxide microsponges have been prepared applying solvent diffusion emulsion technique, which was successful in releasing the drug in a controlled manner with minimal skin irritation (Jelvehgari et al., 2006). A new formulation of 4% hydroquinone entrapped in microsponges reservoir was formulated to slowly release hydroquinone thus, increases the contact time with the treatment and decreases skin irritation (Grimes, 2004). Microsponges delivery system of diclofenac diethylamine was formulated by Osmani et al. (2015) to provide prolonged release for arthritis therapy. Patel et al. (2016) developed ethyl cellulose microsponges formulation for topical delivery of fluconazole for skin fungal infections. In a comparable study, Pande et al. (2015) formulated and in vitro characterized sertaconazole nitrate microsponge as a topical drug delivery system. Nebivolol-loaded microsponges system for healing of diabetic wounds was prepared by Pandit et al. (2017). Another research group prepared silver sulfadiazine loaded microsponge based gel for second degree burns (Kumar and Ghosh, 2017). Microsponges gel imparted prolonged drug release with significant and fast wound healing and closure in diabetic rats. Miconazole nitrate microsponges formulation for diaper rash has been prepared by Gulati et al. (2016). The study was performed using the water soluble form of MCZ, thus it behaves differently from the hydrophobic form (MCZ). In addition, the intended use of the formulation, for diaper dermatitis, hasn't been tested.

In another study, ethyl cellulose based microsponge delivery system for itraconazole vaginal delivery was designed by Katkade et al. (2013). The study represented an approach towards the production of itraconazole vaginal microsponges, but the in vitro antifungal activity and in vivo therapeutic efficacy of the formulation were not evaluated.

In the present work, we are aiming at developing a vaginal microsponges system for MCZ. The proposed formula would increase patient compliance by minimizing possible side effects of MCZ like local irritation, prolong the action of the drug by controlling drug release and increase vaginal retention due to the unique porous structure of the microsponge system which will result in an overall increase in the therapeutic efficacy of the system compared to conventional vaginal products.

2. Materials and method

2.1. Materials

MCZ (generously gifted by Amoun Co., Egypt), Eudragit RS 100 was purchased from Degussa-Rhom GmbH and Co, Germany. Poly vinyl alcohol (PVA) mol wt 88,000 was obtained from Acros Organics New Jersey USA. Dichloromethane, ethanol, potassium dihydrogen phosphate, disodium hydrogen phosphate and sodium lauryl sulphate (SLS) were purchased from (Adwic, El-Nasr Chemical Co., Cairo, Egypt), Carbopol 940 was purchased from (Sigma-Aldrich, Buchs). Gyno-Daktarin® 20 mg/g vaginal cream, Janssen Pharmaceutica NV, Turnhoutseweg 30, B-2340 Beerse, Belgium (the market product). All other chemicals used were of analytical grade.

2.2. Preparation of MCZ microsponges

A modified Quasi emulsion method for microsponges preparation was applied (Graves et al., 2005). To prepare the inner phase, 100 mg Eudragit RS 100 was dissolved in ethyl alcohol, then the drug was added to the solution and dissolved under ultrasonication (CREST, Ultrasonic Corporation, Cortland, New York) at 35 °C for 10 min. The inner phase was poured into 100 mL polyvinyl alcohol solution in water (outer phase). Following 120 min of stirring at 500 rpm, the mixture was filtered to separate the formed microsponges. For optimizing the preparation method, drug:polymer ratio (1:1, 2:1 and 4:1), the amount of PVA (25, 50 and 75 mg) and volume of organic solvent (2.5, 5, 10 mL) were changed, and the characteristics of the prepared microsponges were evaluated. The microsponges were dried in an air-heated oven at 40 °C for 12 h. Composition of the formulae is given in Table 1.

2.3. Characterization of MCZ microsponges

2.3.1. Determination of production yield (%), drug content and entrapment efficiency (EE %)

Using the initial weight of the raw materials and the weight of the obtained microsponge particles, the production yield was calculated. Samples of microsponges (20 mg) were dissolved in 10 mL ethanol under sonication for 20 min at 25 °C. The samples were filtered through 0.45 μm membrane filter and analyzed spectrophotometrically for MCZ content using Shimadzu UV-1650 UV–VIS double beam spectrophotometer, Japan at 273 nm. Drug content and EE % were calculated as given below.

$$\text{Drug content } (\%) = (M_{act}/M_{ms}) \times 100 \quad (1)$$

$$\text{EE } (\%) = (M_{act}/M_{th}) \times 100 \quad (2)$$

where M_{act} is the actual MCZ content in the weighed quantity of microsponges, M_{ms} is the weight of microsponges powder, and M_{th} is the theoretical amount of MCZ in microsponges calculated from the quantity added during preparation.

2.3.2. Particle size determination

The particle size of microsponges was determined using dynamic laser scattering technique (Beckman Coulter LS 13 320 Brea, California, US). The aqueous microsponge suspension was added dropwise to the sample cell attached inside the counter till an obscuration rate of 5% was achieved. Particle size distribution and other statistical parameters were analyzed by the inbuilt software version 3.29.

2.3.3. In vitro drug release of MCZ microsponges

Microsponges (50 mg) were placed in the basket (mesh #230 = 63 μm) of USP apparatus 1. The study was performed in 150 mL acetate buffer pH 4 to mimic the pH of the vagina. Due to the low aqueous solubility of MCZ, 1% SLS was incorporated in the release medium to maintain sink condition. The study was performed at 37 ± 0.5 °C for 6 h at 50 rpm. Aliquots were withdrawn periodically and compensated by adding equal volume of the release medium. The samples were analyzed spectrophotometrically at 273 nm and % drug release versus time plots were constructed.