Preparation and Characterisation of Natamycin: γ-Cyclodextrin Inclusion Complex and its Evaluation in Vaginal Mucoadhesive Formulations

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ABSTRACT: Novel formulations of vaginal bioadhesive tablets were prepared where the natamycin was complexed with γ-cyclodextrin (NT-γCyD) to increase the solubility and stability of NT in aqueous solutions and reduce the side effects of the drug without decreasing antimycotic activity. Favourable interactions between the NT and γCyD and formation of the 1:1 inclusion complex were observed. The MIC_{90} of both NT alone and NT-γCyD complexes were below 0.0313 μg mL^{-1}, suggesting that complexation with γCyD has effectively increased the antimycotic activity of NT, thus indicating the clinical usefulness of NT-γCyD complexes. The sustained drug release of NT was achieved to over 8 h periods by altering the polymer component of formulations which was responsible for differences in water absorption and erosion behaviour of the tablets. Bioadhesion studies have clearly indicated that enhancement of mucoadhesion was achieved by inclusion of Carbopol®934P and by tailoring the ratio of Carbopol®934P in the formulation, a high mucoadhesion to vaginal mucosa can be achieved. Hence, the formation of complex between NT and γCyD and effective combination with polymers attain a bioadhesive and sustained release formulation of NT suitable for vaginal delivery and the effective treatment of *Candida* infections. © 2008 Wiley-Liss, Inc. and the American Pharmacists Association J Pharm Sci 97:4319–4335, 2008

Keywords: natamycin; γ-cyclodextrin; inclusion complex; vaginal drug delivery; bioadhesion; hydroxypropyl methyl cellulose; xanthan gum; Carbopol®934P; *Candida* spp

INTRODUCTION

Candida infection is a common microbial problem in the vulvovaginal tract. Approximately 75% of women will have had a vaginal *Candida* infection during their lifetime, about 40–50% of them will suffer a relapse, and a small percentage will be affected chronically by this infection.[1]

Natamycin (NT) belongs to the family of polyene antifungal antibiotics. The antifungal activities of NT and other amphiphilic polyenes targets the cytoplasmic membrane by interacting primarily with ergosterol, resulting in enhanced leakiness of the fungal cell membrane and in turn cell death.[2]

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NT has been applied topically as a suspension or ointment for skin, ocular or oral candidiasis, as an ointment for dermatomycosis.[3] Importantly, Natamycin is also delivered as a vaginal tablet for the treatment of vaginitis/vaginosis caused by *Candida albicans* and *Trichomonas vaginalis*.[4] The vaginal tablet is usually administered as one vaginal tablet per day for 20 days or 2×1 vaginal tablets for 10 days after the menstrual cycle. Such conventional treatment for vaginitis/vaginosis is limited by poor patient compliance due to its high frequency of administration. NT is poorly soluble in water ($\sim$50 mg L^{-1}) and in acidic solutions, displaying rapid degradation under such conditions. Its poor solubility makes it largely unavailable for medical utility. Korteweg et al.[5] attempted to solubilise the drug by mixing it with a complex polysaccharide. Although the water solubility of the formulation increased dramatically, its antifungal activity decreased to about 1/3 of that of native NT and this formulation was more toxic than NT. The ability to increase the aqueous solubility and stability of NT by cholate formation has shown very limited success.[6] Koontz and Marcy[7] have already shown that the solubility of NT has been increased by complexation of NT and CyD.

Cyclodextrins (CyDs) and their chemically modified CyD derivatives are used in the pharmaceutical field to form inclusion complexes with drug molecules to improve the aqueous solubility of the encapsulated species, to improve their aqueous stability and photostability and to reduce side effects.[8–10] CyDs are cyclic oligosaccharides consisting of at least six D (+) glucopyranose unites covalently linked by α-(1,4) glucosidic bonds.[11,12] The natural CyDs, αCyD, βCyD and γCyD with 6, 7 or 8 glucose units, respectively, possess different cavity size and solubility. The unique molecular structure of CyDs, with hydrophobic internal cavities and a hydrophilic external surface, endows CyDs with the ability to form inclusion complex with various guest molecules.[13] The binding forces of the guest molecule within these inclusion complexes include hydrophobic, van der Waals, hydrogen bonding or dipole interaction.[11,14] CyD has also been reported to enhance the controlled release properties of certain active ingredients.[15] Importantly, while incomplete drug release may occur in cases whereby drug dissolution is limited by the solubility of the drug within the matrix tablets, incorporation of hydrophobic drug-CyD inclusion complexes into hydrophilic matrix tablets may provide a more controlled and complete *in vitro* drug release.[16]

Alternatively, the inclusion of mucoadhesive polymers into vaginal formulations (e.g., gels, tablets) has increased the residence time of the desired drug in the vagina, thereby boosting the efficacy of the treatment.[17,18] Apart from prolongation of drug release at the site of absorption, drug targeting to the affected site can also be realized.[19,20] Mucoadhesive drug delivery systems exploit the useful property of mucoadhesion of certain biopolymers on interaction with mucus that is present at the targeted physiological sites, for example, the vaginal mucosa. Mucus is a mixture of large glycoproteins (mucins), water, electrolytes, epithelial cells, enzymes, bacteria and various other materials depending on the targeted mucosal route. The vaginal epithelium is usually considered as a mucosal surface, albeit the absence of goblet cells and the lack of direct release of mucin, which often characterise mucosal surfaces.[21] Bioadhesive polymers with a high swelling index in aqueous environment are often used to produce controlled release formulations. Commonly employed bioadhesive polymers in pharmaceutical preparations can be derived from synthetic and from natural sources. Examples of bioadhesive polymers include cellulose derivatives such as hydroxypropyl cellulose, hydroxypropyl methylcellulose (HPMC), carboxymethyl cellulose; polyacrylic acid derivatives such as Carbopol®934P (C934P), polycarbophil; and natural gums such as xanthan gum (XG) and guar gum.[22–25]

Here, it is hypothesized that increased solubility of NT will lead to the reduction in NT dose sufficient for producing antimycotic activity may result in formulations with lower toxicity. The full characterisation of NT-γCyD confirmed that formation of inclusion complexes. The development of vaginal tablets containing NT with enhanced bioavailability is therefore warranted for treatment of vaginal *Candida* infections. By employing the NT-γCyD inclusion complexation approach, it is hoped that controlled release and bioadhesive vaginal tablets with improved NT solubility, stability, dissolution properties and reduced side effects can be realised. Accordingly, bioadhesive vaginal tablet containing NT-γCyD complexes present as an admixture with bioadhesive polymers such as XG, HPMC and C934P has been prepared.

MATERIALS AND METHODS

Materials

Natamycin (M_w: 666 Da) was obtained from Shenzhen SED Industry Co., Ltd., China.