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Comparative analysis of binary and ternary cyclodextrin complexes with econazole nitrate in solution and in solid state

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ABSTRACT

The aim of this work was to investigate in-depth interactions of econazole nitrate (ECN), a very poorly water-soluble antifungal agent, with different β-cyclodextrin (βCD) derivatives, and to evaluate the potential synergistic effect of suitable third compounds (L-amino acids, citric acid, hydrophilic polymers). Phase-solubility studies showed the formation of equimolar complexes with all tested CDs, and indicated sulfobutyl-βCD (SBEβCD) as the best complexing and solubilizing agent for ECN, followed by hydroxypropyl-βCD (HPβCD). 1D and 2D ^{1}H NMR studies demonstrated the actual formation of inclusion complexes of 1:1 mol:mol stoichiometry, and gave insight about different inclusion modes of ECN molecule into the CD cavity, simultaneously existing in solution. Among the different tested ternary systems, only those with citric acid (CA) enabled a significant increase in complexing and solubilizing ability towards the drug with respect to the binary ones, indicating a synergistic effect between SBEβCD and CA and the formation of highly soluble ternary complexes, which was further supported by NMR studies. Solid equimolar binary and ternary systems of ECN, CDs and CA were prepared by co-grinding in high energy vibrational micro-mills and characterized by differential scanning calorimetry, X-ray powder diffractometry and *in vitro* dissolution studies. In the case of binary systems, total sample amorphization, indicative of strong solid state interactions and possible inclusion complex formation, was obtained only for co-ground products with HPβCD and SBEβCD, but they both presented a dissolution profile typical of a supersaturated system, with a limited improvement of drug dissolution efficiency (8.3 and 22.13 times, respectively). On the contrary, the ternary ECN/SBEβCD/CA co-ground product presented superior dissolution properties, increasing the ECN dissolution efficiency of 66.62 times, clearly having the best potential for further development of a novel ECN delivery system for efficient delivery of the drug to the oral cavity, thus improving the therapy of oral candidosis.

1. Introduction

Candidosis is by far the most common fungi infection of the human oral cavity, which manifests in a variety of clinical aspects. The infections caused by the yeast *Candida albicans* and several related species range from relatively trivial conditions, such as oral thrush, to systemic super-infections in immunocompromised patients, with mortality of 30–50%. Nowadays, oral candidosis became an emerging medical condition, as a consequence of greater use of invasive surgical procedures, radiotherapy of head and neck carcinoma, common endocrine disorders such as diabetes mellitus, adverse effects of immunosuppressive broad-spectrum antibiotics and inhaled corticosteroids therapies [1,2]. However, the main key of the increased incidence of oral candidosis is in the escalation of HIV-infection, where more than 90% of the patients are afflicted with this pathology [3]. Despite the availability of several effective antimycotics for the treatment of oral candidosis, failure of therapy is not uncommon, due to the unique properties of the oral cavity, where the flushing effect of saliva and the cleansing action of the oral musculature tend to reduce the local drug concentration to sub-therapeutic levels [4]. Therefore, the development of novel, more effective formulations should be aimed to overcome such drawback and achieve an efficient local drug delivery, enabling maintenance of drug concentration in the salivary fluid higher than the minimal inhibitory concentration (MIC) over an extended period of time [5].

The two most frequently administered azole antimycotic agents in the treatment of oral candidosis are fluconazole and itraconazole, which are readily absorbed through the gut, being effectively delivered by oral administration. Furthermore, fluconazole is secreted in high levels in saliva, making this agent particularly suitable for treating oral infections. Unfortunately, in recent years Candida

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resistance to such drugs is rising, resulting in the need to introduce other azole antimycotics into the therapy of oral candidosis [3].

Econazole (EC) is an imidazole antifungal agent practically insoluble in water, and therefore it is commonly used in pharmaceutical formulations as nitrate salt (ECN), which is somewhat more soluble. After topical application, ECN transmucosal and transdermal absorption is not significant, with less than 1% of an applied dose absorbed in the blood [6]. Therefore ECN could be useful for a topical therapy of oral candidosis. However, before the development of a suitable ECN buccal delivery system, able to assure ECN concentrations significantly higher than MIC for a prolonged time period, the poor drug solubility issue should be addressed. In fact, the limited volume of saliva, acting as the dissolution medium in the mouth must be considered, which commonly ranges, in healthy adult subjects, from 0.6 to 1.2 mL [7].

Previous studies showed that both dissolution properties and, consequently, microbiological activity of EC can be improved by complexation with natural cyclodextrins (CDs), particularly with β-CD [8]. However, EC/βCD complex showed a modest release from a chewing gum formulation, of only 2.3% of initial drug content after 30 min [9]. This result might be related to the fact that the βCD solubilizing efficiency is highly dependent on the presence and type of excipients. For example, the presence of 0.05 M phosphate buffer reduced the βCD solubilizing efficiency, resulting in complexes with limited aqueous solubility [10]. Furthermore, the obtained complexes showed a significant haemolytic and toxic effect on TR146 cell layers [10]. Among natural CDs, α-CD has the highest solubilizing efficiency for EC, which can be further increased through addition of different hydroxyl acids, due to ternary complex formation [11,12].

The aim of this paper was focused to suitably increase the ECN solubility in simulated saliva, in view of the future development of an effective mucoadhesive buccal formulation of the drug. With this purpose, we examined the performance of a series of chemically modified βCD-derivatives (hydroxypropyl-βCD (HPβCD), hydroxyethyl-βCD (HEβCD), sulfobutyl-βCD (SBE-βCD), and water soluble βCD-epichlorohydrin polymer (EPIβCD)), which are more soluble and have more acceptable toxicological profiles compared to the parent βCD [13]. Phase solubility studies of the drug in simulated saliva in the presence of the different βCD-derivatives were performed, in order to investigate the influence of the substituent type on the CD solubilizing and complexing efficiency towards ECN, and to find the most effective drug carrier. After this initial screening, the complexing and solubilizing potential of the most efficient βCD-derivative was further enhanced by addition of a suitable third compound to the complexation medium. For this purpose, the effectiveness of some amino acids (L-glycine and L-lysine), hydrophilic polymers (HPC and PVP) and citric acid has been tested. Both 1D and 2D ^{1}H NMR spectroscopic studies were carried out in order to clarify the interaction mechanism in solution and determine the structures and the stoichiometry of the complexes formed. Binary and ternary complexes in the solid state were prepared by co-grinding the related physical mixtures in a high energy vibrational micromill. Previous investigations showed that the mechanochemical treatment performed by the co-grinding procedure could lead to formation of drug/CD inclusion complexes in the solid state [14–16]. Compared to other methods for inclusion complex preparation in the solid-state, co-grinding offers the advantages to be a fast and simple method not requiring use of organic solvents, whose elimination from the final product could be complicated and costly, thus representing an economically and environmentally desirable technology [17]. The solid state properties of the products have been characterized by differential scanning calorimetry and X-ray powder diffractometry, while their dissolution behaviour in simulated saliva has been assessed by mimicking the *in vivo* conditions, in order to rationally select the most effective product for further development of an advanced dosage form aimed to improve the therapy of oral candidosis.

2. Materials and methods

2.1. Materials

Econazole (1-[2-(4-chlorophenyl)methoxy]-2-(2,4-dichlorophenyl)ethyl) 1H-imidazole mononitrate, ECN) was kindly donated by Italfarmaco (Italy). The list of cyclodextrin derivatives used in this study comprised hydroxypropyl-β-cyclodextrin (HPβCD), hydroxyethyl-β-cyclodextrin (HEβCD), both with an average substitution degree per anhydroglucose unit of 0.6, kindly donated by WackerChemie (Germany); water-soluble β-cyclodextrin-epichlorohydrin polymer (EPIβCD, mean MW 4500) purchased from Cyclolab (Hungary) and sodium salt of sulfobutyl-β-cyclodextrin with a substitution degree of 0.9 (SBEβCD) obtained by CyDex, Inc. (USA). Amino acids L-glycine (L-glc) and L-lysine (L-lys) were obtained from Euphar group s.r.l. (Italy). Hydrophilic polymers used were hydroxypropyl cellulose (HPC; Klucel EXF, Signet Chemical Corporation Pvt. Ltd., India), and polyvinylpyrrolidone (PVP, Kollidone K25, BASF, Germany). Anhydrous citric acid (CA), deuterium oxide (D_2O) with minimum isotopic purity of 99.96% and HPLC grade methanol were obtained from Sigma–Aldrich (Italy). Simulated saliva solution was prepared by dissolving 2.38 g Na_2HPO_4, 0.19 g KH_2PO_4, 8.00 g NaCl in 1 L of distilled water and adjusting pH to 6.75 by the use of orthophosphoric acid. All others chemicals and solvents used in this study were of analytical reagent grade.

2.2. Phase solubility studies

An excess amount of ECN (20 mg) was added to 5 mL of simulated saliva (pH 6.75) containing the increasing amounts of cyclodextrin tested, in concentrations ranging from 0 to 25 mM (binary systems). The influence of selected amino acids (L-glycine and L-lysine) and hydrophilic polymers (HPC and PVP) in concentrations of 0.5 and 1.0% (w/v) on the drug solubility was evaluated by adding their accurately weighted amounts into 5 mL of simulated saliva solution containing 20 mg of ECN. Ternary systems were prepared by adding 1% (w/v) of the polymers or equimolar amount of the CA to the selected binary drug/cyclodextrin systems, while the quaternary systems were prepared by combining the drug, selected cyclodextrin, 1% (w/v) of the polymer and equimolar quantity of the CA. The prepared samples in sealed glass containers were thermostated at 25 °C and vigorously stirred for 72 h, until complexation equilibrium was reached. Aliquots of the samples were filtered through 0.45 μm Millipore membrane filter and the drug concentration in the filtrates was analyzed by HPLC. It was verified that ECN did not absorb to the filters used for separation of the undissolved solids. Phase solubility diagrams were obtained by plotting the apparent drug solubility against cyclodextrin concentration in the sample. The apparent stability constants (Ks) were calculated according to Higuchi and Connors [18], by using the slope of the straight portion of the phase solubility diagram and ECN solubility in simulated saliva in the absence of CD (s_0):

$$K_s = \frac{\text{slope}}{s_0 \times (1 - \text{slope})} \quad (1)$$

2.3. HPLC assay of ECN

The drug HPLC assay was performed using a Merck Hitachi Elite Lachrom apparatus equipped with a L2130 model isocratic pump,