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Inclusion complexes of chlorzoxazone with β- and hydroxypropyl-β-cyclodextrin: Characterization, dissolution, and cytotoxicity

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ABSTRACT

This study aimed to improve the water solubility and reduce the toxicity of chlorzoxazone via complexation with β-cyclodextrin (β-CD) and hydroxypropyl-β-cyclodextrin (HP-β-CD). Inclusion complexes between chlorzoxazone and the two cyclodextrins (CDs) were prepared by freeze-drying method. Formation of the complexes was confirmed by FT-IR, PXRD, ^{1}H NMR, DSC, and SEM. The water solubility and dissolution rates of chlorzoxazone were significantly increased by complexation with the two CDs. Preliminary in vitro cytotoxicity tests showed that the complexes are less toxic to normal liver cells than free chlorzoxazone. In general, the HP-β-CD complex exhibited better dissolution properties than the β-CD complex in various dissolution media. Therefore, the HP-β-CD complex can be used to design novel formulations of chlorzoxazone.

1. Introduction

Chlorzoxazone (CZX, Fig. 1a) is a synthetic drug with various pharmacological actions. This drug is regularly used as a central nervous system-acting muscle relaxant to treat muscle spasms (Attia, Ramsis, Khalil, & Hashem, 2012) and provide pain relief. CZX has been proven to present certain curative effects on systemic mastocytosis, conjunctivitis, and ulcerative colitis (Gnanasambandan, Gunasekaran, & Seshadri, 2014); it can also be used to treat children's mental dysplasia caused by central nervous system lesions. Several researchers have reported a number of CZX applications (Feil et al., 2013; Hopf et al., 2011; Shaik & Mehvar, 2011).

Despite the many benefits of the drug, the biomedical applications of CZX are still limited by its disadvantages, which include low water solubility and high toxicity. Due to its poor water solublility, oral solid preparation has been the main form of CZX in clinical application. Thus, to improve the solubility and dissolution of CZX is a problem requiring to be solved urgently. Previous studies reported use solid dispersion technique or cosolvents solubilization method to enhance the solubility or dissolution of CZX (Chen & Frank, 1983; Swati, Asawaree, Aniruddha, & Bhanudas, 2013). However, the dissolution of the product still has great room for improvement.

High toxicity of CZX is the other limitation for clinical application. Clinical studies have shown that CZX exhibits a particular degree of liver toxicity after it was applied with a combination of acetaminophen (Parafon Forte) for several months, and two deaths involving hepatic failure have been reported (Powers, Cattau, & Zimmerman, 1986). Thus, in view of the above shortcomings, to employ other methods to increase water solubility and dissolution rate, and decrease toxicity of CZX is a crucial and meaningful endeavor.

Cyclodextrin (CD) complexation technology has often proved to be the most successful in improving the solubility and dissolution of poorly soluble drug. CDs (Fig. 1b) generally possess a truncated

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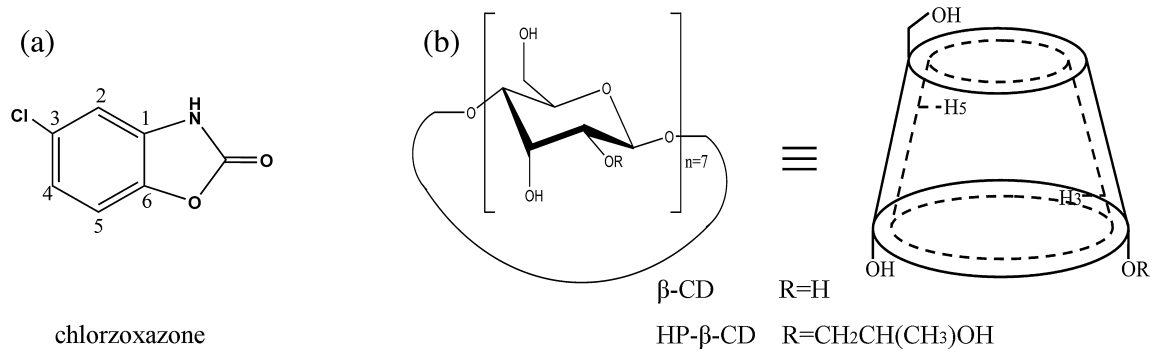

Fig. 1. Structures of (a) chlorzoxazone, and (b) β-cyclodextrin, and hydroxypropyl-β-cyclodextrin.

conical shape as well as structures with a hydrophobic cavity, hydrophilic ends, and an external region. They present important functions in the field of supramolecular chemistry because several types of guest molecules may be accommodated by the CD cavity (Delvalle, 2004; Saenger, 1980; Szejtli, 1998). In particular, CDs are believed to enhance the physicochemical and pharmacodynamic properties, including stability, solubility, dissolution rate, and drug bioavailability, of their guest molecules (Bekers, Uijtendal, Beijnen, Bult, & Underberg, 1991; Duchene & Wouessidjewe, 1990). To the best of our knowledge, no scientific study about the effect of CDs on increasing the solubility and dissolution rates of CZX has yet been published.

This work aimed to improve the water solubility and reduce the toxicity of CZX via complexation with β-cyclodextrin (β-CD) and hydroxypropyl-β-cyclodextrin (HP-β-CD, Fig. 1b). Firstly, drug/CD interactions in solution were investigated by phase solubility studies, the solid complexes were prepared and characterized by Fourier-transform infrared spectroscopy (FT-IR), powder X-ray diffraction (PXRD), ^{1}H nuclear magnetic resonance spectroscopy (^{1}H NMR), differential scanning calorimetry (DSC), and scanning electron microscopy (SEM). In addition, molecular models of the two complexes were calculated to verify the geometrical configurations of the complexes from experimental results. Moreover, to investigate the possible dissolution properties of the complexes in the stomach and intestine, pure water and aqueous solutions of pH 1.2 and 6.8 were used as dissolution media in dissolution tests. Finally, the inhibitory effects of free CZX and the complexes on liver LO2 cell growth were also investigated to evaluate decreases in the toxicity of CZX. The CD inclusion complexes exhibit great potential for future biomedical applications.

2. Materials and methods

2.1. Materials

CZX (purity > 99%), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT), and dimethyl sulfoxide (DMSO) were purchased from Sigma–Aldrich Co., LLC. (Shanghai, China). HP-β-CD and β-CD (BR purity > 99%) were obtained from Chengdu Kelong Chemical Co., Ltd. (Chengdu, China). The human liver cell line LO2 was purchased from the American Type Culture Collection (Manassas, VA, USA). Other reagents and chemicals used were of analytical reagent grade. Tri-distilled water was used throughout the experiment.

2.2. Synthesis of the inclusion complex

Inclusion complexes of CZX with β-CD and HP-β-CD were prepared by the freeze-drying method at a 1:1 molar ratio. CZX dissolved in methanol was added drop-wise to dilute aqueous solutions of β-CD and HP-β-CD. The mixed solution was stirred at a controlled temperature (40 ± 1 °C) for 5 h and then heated to 60 °C to remove the organic solvent. The obtained solution was filtered, gradually cooled down to room temperature, and then placed in a refrigerator at −20 °C. The fully frozen solution was dried in a vacuum freeze dryer, and the resulting solid complexes were collected.

2.3. Phase solubility studies

Phase solubility studies were performed according to the methods described by Higuchi and Connors (Higuchi & Connors, 1965). Excess amounts of CZX (30 mg) were added to aqueous solutions containing increasing amounts of β-CD or HP-β-CD (0–20.00 mM). The mixtures were placed in an ultrasonic bath for 15 min and then left to stand for 7 d at 22, 27, 32 and 37 °C. After equilibrium was achieved, each solution was filtered through a 0.45 μm Millipore filter, properly diluted, and then detected by a TU-1901 ultraviolet detector (PERSEE, China) at 280 nm to calculate the residual concentration of CZX. All experiments were performed in triplicate.

The apparent stability constants (K_C, L/mol) of the two complexes were calculated by analyzing their phase solubility curves (Higuchi & Connors, 1965) using Eq. (1):

$$K_c = \frac{\text{slope}}{S_0(1 - \text{slope})} \quad (1)$$

where S_0 is the solubility of CZX in tri-distilled water in the absence of CDs. The change in Gibbs' free energy (ΔG) of the complexation process was calculated according to the equation $\Delta G = -RT\ln K$ where R is the universal gas constant (8.314 J/mol K) and T is the experimental operating temperature (295, 300, 305, or 310 K). The Van't Hoff equation (Eq. (2)) was employed to calculate enthalpy (ΔH) and entropy (ΔS) changes in the two inclusion complexes.

$$\ln K = -\frac{\Delta H}{RT} + \frac{\Delta S}{R} \quad (2)$$

2.4. Characterization of the complex

Characterization was performed to confirm the formation of complexes and demonstrate their possible configurations. The FT-IR spectra of the CDs, free CZX, their physical mixtures, and their complexes were recorded by a Nicolet 6700 FT-IR spectrometer (Thermo Fisher Scientific, USA) over a scanning range of 4000–400 cm^{-1}. Each sample was prepared into slices of 1 mg of sample in 100 mg of KBr.

Laboratory PXRD patterns for samples were collected at 25 °C using an X'Pert PRO diffractometer (PANalytical, Holland) with an X'celerator detector and Cu$K\alpha_1$ radiation (λ = 1.54056 Å, generator setting: 40 kV, 40 mA). Diffraction data were collected over the