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Telmisartan complex augments solubility, dissolution and drug delivery in prostate cancer cells

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ABSTRACT

Telmisartan (TEL) requires superior bioavailability in cancer cell compartments. To meet these challenges, we have synthesized a 2-HP-β-CD-TEL complex with stability constant (K_c) of 2.39×10^{-3} mM. The absence in the FTIR spectrum of 2-HP-β-CD-TEL complex of the characteristic peaks of TEL at 1699 cm^{-1} (carboxylic acid) and 741 and 756 cm^{-1} (1,2-disubstituted benzene ring vibrations), is indicative of the encapsulation of TEL in the 2-HP-β-CD cavity. DSC and PXRD also confirmed the synthesis and amorphous structure of complex. The interaction of TEL with 2-HP-β-CD was examined by NMR and 2D-ROESY which affirms the encapsulation of TEL in the 2-HP-β-CD cavity in at least two orientations with equal binding energies. The complex also exhibited its superiority in both *in vitro* release and cytotoxicity experiments on prostate cancer, PC-3 cells as compared to free drug. These data warrant an in depth *in vivo* to scale-up the technology for the management of prostate cancer.

1. Introduction

It is known that 70% of all new chemical entities entering drug discovery programs are not sufficiently soluble in physiological medium to allow consistent gastrointestinal absorption of high magnitude to ensure further pharmacodynamic activities. The solubility of a drug in gastrointestinal tract is governed by multiple factors and is inherently a complex phenomenon often resulting in erratic absorption of poorly soluble drugs. Therefore, an improvement in the dissolution rate of the drug is thought to be a key factor for improving the pharmacokinetic and pharmacodynamic activities. Nanonization of lipophilic drugs by methods such as crystal modification, size reduction, pH modification and amorphization have been reported to enhance solubility and thereby bioavailability of drugs (Jain et al., 2013). Thus, *state-of-the-art* nanonization techniques much earlier in the drug discovery and development cascade are warranted to increase the number of therapeutic agents available for clinical studies.

Angiotensin II receptor blockers (ARBs) have been categorized as antihypertensive agents (Billecke & Marcovitz, 2013). In addition, ARBs have the potential to inhibit the growth of several types of cancer cells (Abali et al., 2002). Telmisartan (TEL), a component in the armamentarium of ARBs inhibits 12-O-tetradecanoylphorbor-13-acetate (TPA)-induced cell proliferation stronger than candesartan (Ozeki et al., 2013). TEL has peroxisome proliferator-activated receptor (PPAR)-γ activation activity. It causes significant growth inhibition in the prostate cancer cells in a dose- and time-dependent manner (Matsuyama et al., 2010). Thus, TEL could be a new potent chemical entity for the prevention and treatment of human cancers. However, poor aqueous solubility (0.078 mg/ml) and suboptimal oral bioavailability (>50%) (Marasini et al., 2013; Stangier et al., 2000) consequently appeal for development of a clinically viable oral dosage form which can offer superior pharmacokinetic profile and high therapeutic concentration in cancer cell compartments.

We have successfully synthesized cyclodextrin (CDs) complexes of anticancer drugs and reported high therapeutic index in human cancer cells (Chauhan et al., 2013; Madan et al., 2010, 2012). Biocompatible CDs have been approved by Food and Drug Administration for human consumption. CDs have bucket shaped structures which enable pharmaceutical scientists to entrap a wide variety of lipophilic drugs (Sangalli et al., 2001). Structurally, CDs

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consisting of six, seven, or eight glucopyranose units, namely, α-, β-, and γ-CD, covalently linked by α,1-4-glycosidic bonds to form the macromolecule (Davis & Brewster, 2004). The unique properties of β-CD allow it to form a complex with a wide variety of drugs by various forces of attraction. However, the limited solubility of β-CD in the aqueous phase (18.5 mg/ml) hampers its applications in drug delivery (Stella & Rajewski, 1997). In addition, the rigid β-CD structure is acquiescent to crystallization upon complexation. However, 2-hydroxypropyl-β-cyclodextrin (2-HP-β-CD), a β-CD analog modified with a 2-hydroxypropyl unit is known to produce more wettable amorphous compounds with expanded water solubility and complexing power (Kim, 2013). 2-HP-β-CD tenders a significant advantage over β-CD as its solubility in the aqueous phase is >500 mg/ml (Lin, Chean, Ng, & Chan, 2000).

Thus, to improve the physicochemical and pharmacodynamic characteristics of TEL, we have synthesized a 2-HP-β-CD-TEL complex using inclusion chemistry via the cycloencapsulation mode. The 2-HP-β-CD-TEL complex was characterized both in solution and solid state by phase solubility analysis, Fourier-transform infrared (FTIR) spectroscopy, differential scanning calorimetry (DSC), powder X-ray diffraction (PXRD), scanning electron microscopy (SEM), and 1D/2D NMR (ROESY) spectroscopy. Also the 2-HP-β-CD-TEL complex was modeled by *in silico* docking and molecular dynamics simulations to decipher the binding poses and to estimate the relative binding affinities. Further 2-HP-β-CD-TEL complex was examined for cytotoxic activity in prostate cancer, PC-3 cells. Standard colorimetry based MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] cell proliferation assays was used to assess the delivery and pharmacodynamic efficacy in prostate cancer cells of the complex compared to the free drug.

2. Materials and methods

2.1. Materials

Telmisartan (TEL, molecular weight ~514 Da, purity ~98%) was a gift sample from Glenmark Pharmaceuticals, India. 2-Hydroxypropyl-betacyclodextrin (2-HP-β-CD, molecular weight ~1541.54 Da) was purchased from Chemsworth, Surat, India. D_2O (99.9% purity) and dimethyl sulfoxide-d_6 (DMSO-d_6) (D, 99.9% + 1%, v/v TMS) were purchased from Cambridge Isotope Laboratories, Inc. NaOD (40 wt% in D_2O, 99+ atom% D) was purchased from Acros Organic. All other chemicals used were of highest analytical grade and used without further purification.

2.2. Cell culture

Human prostate cancer cell line (PC-3) was maintained in 5% CO_2 and 95% air at 37 °C using Dulbecco's modified Eagle's medium (DMEM) (Biologicals, Israel) supplemented with 5% fetal calf serum. All experiments were performed with asynchronous populations in exponential growth phase (24 h after plating) (Li & Sarkar, 2002).

2.3. Phase solubility analysis

Phase solubility assay was implemented to assess the stoichiometry of the 2-HP-β-CD-TEL complex in the aqueous phase (de Melo, Grillo, Rosa, & Fraceto, 2008). Briefly, TEL (20 mg) was suspended separately in 10 ml of phosphate buffer saline (PBS, pH ~7.4) containing 2-HP-β-CD at concentrations ranging from 2 to 32 mM. Next, samples were stirred in an orbital shaker (150 rpm) for five consecutive days at 37 ± 1 °C. After equilibration, the samples were passed separately through a 0.22-μm membrane filter (MDI, India), and the absorbance was read at 298 nm using an UV/visible spectrophotometer (Shimadzu, Kyoto, Japan) (Kondawar, Kamble, Raut, & Maharshi, 2011). The apparent stability constant was calculated from the slope of the phase-solubility diagram using Eq. (1):

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

where K_c is the apparent binding/stability constant and S_o is the solubility of the drug in the absence of cyclodextrin.

2.4. Synthesis of solid complexes using inclusion chemistry

Solid complex of TEL with 2-HP-β-CD was synthesized by mixing TEL with 2-HP-β-CD in aqueous phase (pH ~10.0) in 1:1 molar ratio. The resultant solutions were stirred for 24 h in an orbit shaker (150 rpm) at 37 ± 1 °C. Subsequently, the solutions were lyophilized and collected as solid complex. We also prepared physical mixture of TEL with 2-HP-β-CD in a 1:1 molar ratio by mixing the individual components and passing them through sieve #100.

2.5. Characterization of solid complexes

2.5.1. Fourier-transform infrared (FTIR) spectroscopy

The first characterization of the complex of TEL with 2-HP-β-CD was done using FTIR spectroscopy. The spectra of TEL, 2-HP-β-CD, the physical mixture of TEL with 2-HP-β-CD and the complex, 2-HP-β-CD-TEL were recorded in a Perkin Elmer IR spectrophotometer. KBr was used to prepare the sample pellet (2 mg sample/200 mg KBr) at a force of 40 psi for 4 min using a hydrostatic press. The samples were scanned between 4000 and 400 cm^{-1} with a resolution of 4 cm^{-1}.

2.5.2. Differential scanning calorimetry (DSC)

DSC was used to confirm the synthesis of the complex in the solid state. Characteristic endothermic peaks of TEL, 2-HP-β-CD, physical mixture of TEL with 2-HP-β-CD and the complex, 2-HP-β-CD-TEL were recorded using a Mettler-Toledo differential scanning calorimeter. Nitrogen was used as the carrier gas at a flow rate of 45 ml/min. Thermograms were recorded at a heating rate of 20 °C/min in the temperature range of 30–300 °C with 10 mg of sample.

2.5.3. Powder X-ray diffraction pattern (PXRD)

The crystalline configuration of TEL, 2-HP-β-CD, physical mixtures of TEL with 2-HP-β-CD and the complex, 2-HP-β-CD-TEL were studied using a Rigaku, Rotaflex, RV 200 (Rigaku Corporation, Japan) X-ray diffractometer with Ni filtered, Cu Kα radiation, at a voltage of 60 kV and a current of 45 mA. The scanning rate employed was 2°/min over the diffraction angle (2θ) range.

2.5.4. Scanning electron microscopy (SEM)

TEL, 2-HP-β-CD, the physical mixture of TEL with 2-HP-β-CD and the 2-HP-β-CD-TEL complex were examined by a scanning electron microscope (SEM) to visualize the surface topography. Samples were prepared by preparing the film on an aluminum stub. The stubs were then coated with gold to a thickness of 200–500 Å under an argon atmosphere using a gold sputter module in a high vacuum evaporator. The coated samples were scanned, and photographs were taken with a SEM (Jeol-1761, Cambridge, UK) camera.

2.5.5. Nuclear magnetic resonance (^{1}H NMR) spectroscopy

^{1}H NMR spectra were recorded on a Bruker DPX 300 MHz spectrometer to analyze the chemistry of the complex. The solution of TEL (6.0 mM) was prepared in deuterated dimethyl sulfoxide (DMSO-d_6) while 2-HP-β-CD-TEL complex and 2-HP-β-CD (6.0 mM) were prepared separately in deuterated water (D_2O) and then transferred to NMR tubes. The probe temperature was