Journal of Pharmaceutical and Biomedical Analysis 117 (2016) 453–463

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

journal homepage: www.elsevier.com/locate/jpba

The influence of hydroxypropyl-β-cyclodextrin on the solubility, dissolution, cytotoxicity, and binding of riluzole with human serum albumin

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ARTICLE INFO

Article history:
Received 1 August 2015
Received in revised form
26 September 2015
Accepted 29 September 2015

Keywords:
Riluzole
Hydroxypropyl-β-cyclodextrin
Human serum albumin
Inclusion complex
Solubilization
Influence

ABSTRACT

Cyclodextrin-related host–guest encapsulation is fundamental to modulate the solubility of riluzole (RLZ), promoting its potential pharmaceutical applications. The supramolecular interaction of RLZ and hydroxypropyl-β-cyclodextrin (HP-β-CD) was examined through FT-IR spectroscopy, DSC-TGA, PXRD, ^{1}HNMR, 2D ROESY, ssNMR, and SEM. The HP-β-CD/RLZ inclusion complex was formed at a molar ratio of 1:1. The stability constant (K = 2327 M^{-1}) and the corresponding thermodynamic parameters were ascertained through phase solubility studies. The water solubility and dissolution rate of RLZ notably increased in the presence of HP-β-CD, whereas the inclusion complex did not increase the RLZ toxicity toward the LO2 cell line. The influence of HP-β-CD on RLZ–human serum albumin (HSA) binding was investigated via fluorescence spectroscopy. Fluorescence quenching of HSA by RLZ in the presence and absence of HP-β-CD were both static quenching. Data analysis showed that the addition of HP-β-CD weakened the quenching and binding of RLZ with HSA but did not affect the binding site and binding force between RLZ and HSA. Furthermore, molecular models were generated to determine the binding site between HSA and RLZ, and these models were consistent with the experimental data.

1. Introduction

Cyclodextrins (CDs) are cyclic oligosaccharides composed of six (α-CD), seven (β-CD), and eight (γ-CD) or more (α-1,4)-linked α-D-glucopyranose units. CDs exhibit a hydrophobic interior cavity and a hydrophilic exterior surface and can encapsulate various organic or inorganic molecules through the formation of inclusion complexes [1]. These inclusion complexes can remarkably improve the physical, chemical, and biological properties of guest compounds [1,2]. This interesting property enables CDs to be successfully used as drug carriers [3,4], enzyme mimics [5], separation reagents [6], and photochemical sensors [7], etc. Recently, CDs were widely used as complexing agents to enhance the stability, aqueous solubility, dissolution rate, and bioavailability of drug molecules [8–10].

Most drugs are carried from their absorption sites to their action and elimination sites by blood circulation. Human serum albumin (HSA), the most abundant protein in the blood, plays a central role in the pharmacodynamic and pharmacokinetic properties of drugs owing to its capability to bind and transport drugs and participate in drug absorption, distribution, and metabolism [11,12]. Over the years, various studies on the interactions between HSA and small biologically active molecules have been reported [13–15]. However, few studies have focused on drug–HSA binding under the influence of pharmaceutical materials, such as CDs [16,17]. CD, as a carrier molecule, can transport drug compounds through slow and sustained release. Understanding the pharmacokinetics of drugs in CD-encapsulated form and learning the mechanism of medicines at the protein level are essential.

Riluzole (2-amino-6-trifluoromethoxy benzothiazole, RLZ) (Fig. 1a) is a Na^+-channel-blocking medication that can stabilize Na^+ channels and inhibit glutamate release. RLZ exhibits strong neuroprotection, anticonvulsant and antidepressant effects, and sedative properties [18,19], which is mainly used in patients with amyotrophic lateral sclerosis.

However, RLZ is mainly orally administered in spite of its limited aqueous solubility, raising difficulties in its application in dosage forms [20,21]. CDs are a kind of common and efficient host molecules in inclusion reaction, through the non-covalent encapsulation of guest molecules to obtain water-soluble supramolecular molecules. We previously reported the inclusion complexation of RLZ with β-cyclodextrin (β-CD) and 2,6-di-*O*-methyl-β-cyclodextrin (DM-β-CD) [22], but their effects on the

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http://dx.doi.org/10.1016/j.jpba.2015.09.033
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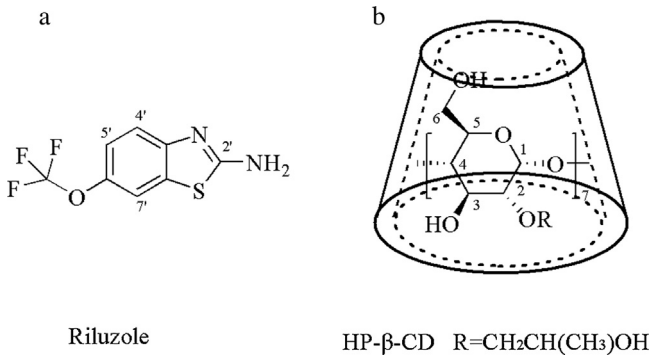

Fig. 1. Chemical structures of (a) RLZ and (b) HP-β-CD.

solubility of RLZ were limited, and the toxicity of β-CD and DM-β-CD through injection limits their further application in pharmaceutical formulations [23,24]. 2-Hydroxypropyl-β-cyclodextrin (HP-β-CD, Fig. 1b) has attracted growing research interest owing to its low toxicity, satisfactory inclusion ability and capacity to improve the drug physicochemical property of drugs [25,26].

The present study aimed to prepare an inclusion complex of RLZ with HP-β-CD to enhance the solubility and dissolution rate of RLZ, evaluate the cytotoxicity of the inclusion complex, and investigate the influence of HP-β-CD on RLZ–HSA binding. Stoichiometry, stability constants, and thermodynamic parameters of the HP-β-CD/RLZ system were determined via phase solubility studies. The inclusion complex was prepared through vacuum drying method, and various analytical techniques were employed to characterize the inclusion complex. The binding interaction of HSA with RLZ in the absence and presence of HP-β-CD, including the binding mechanisms, binding constants, binding sites, and relative thermodynamic parameters, was investigated using fluorescence methods. Additionally, molecular modeling of HSA with RLZ was performed to obtain the molecular geometry.

2. Materials and methods

2.1. Materials

HP-β-CD (MS = 1.0, purity ≥ 98%) was purchased from Best Reagent Co., Ltd. (Chengdu, China). RLZ (FW = 234.20, purity ≥ 98%) was obtained from Yuancheng Saichuang Technology Co., Ltd. (Hubei, China). HSA (fatty acid-free), dimethyl sulfoxide (DMSO), and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were purchased from Sigma (Sigma–Aldrich, Inc.). HSA was dissolved in phosphate-buffered saline (PBS) (50 mM, pH 7.4) at a concentration of 2.0×10^{-5} M, which was determined by ultraviolet spectrophotometry with an extinction coefficient of 36,500 M^{-1} cm^{-1} at 280 nm [27]. The HP-β-CD stock solution was prepared in PBS at 0.1 M. RLZ was dissolved in anhydrous ethanol to obtain a concentration of 5.0×10^{-3} M. Triple-distilled water was used throughout the experiment, and solutions were stored at 0–4 °C. All chemical products were of analytical grade and used without further purification.

2.2. Preparation of solid inclusion complex of RLZ with HP-β-CD

The RLZ inclusion complex with HP-β-CD was prepared at a molar ratio of 1:1 through vacuum drying technique. HP-β-CD (1.54 g) was completely dissolved in 10 ml of water and stirred at 40 °C. RLZ (0.23 g) was dissolved in anhydrous ethanol at 40 °C and added dropwise to the solution. The mixture was then magnetically stirred for 4 h. The resulting solution was filtered to remove unreacted riluzole and vacuum dried at 40 °C to collect the final inclusion complex.

The HP-β-CD/RLZ physical mixture was prepared by mixing RLZ and HP-β-CD at a molar ratio of 1:1 in a vortex mixer for 5 min to obtain a homogeneous blend.

2.3. Experimental methods

2.3.1. Phase solubility studies

Phase solubility studies were performed as described by Higuchi and Connors [28]. An excess amount of RLZ was added to aqueous solutions containing increasing concentrations of HP-β-CD ranging from 0 mM to 20 mM. Then the flasks were shaken for 7 days at 25 °C. During those days the suspensions were sonicated for 4 h/day, and after the equilibrium was reached, the suspensions were filtered through a 0.45 μm Millipore filter. The RLZ concentration in the filtrate was properly diluted and measured at 261 nm using a UV–vis spectrophotometer (TU-1901, Purkinje General Instrument, China). The experiments were repeated three times.

2.3.2. Inclusion complex characterization

The loading efficiency (LE) of RLZ in the HP-β-CD/RLZ inclusion complex was calculated using Eq. (1):

$$\mathrm{LE} = \frac{m_1}{m_2} \times 100\% \quad (1)$$

where m_1 is the amount of drug loaded in the HP-β-CD/RLZ inclusion complex, and m_2 is the entire amount of the HP-β-CD/RLZ inclusion complex. The residual water content of the inclusion complex was determined by SFY-3A Trace moisture meter (Hainuoyiqi Co., Ltd., Shandong, China) using Karl–Fischer method, and the residual ethanol content was determined with gas chromatography (6890A, Agilent Technologies, America).

Fourier transform infrared (FT-IR) spectroscopy, differential scanning calorimetry (DSC), powder X-ray diffraction (PXRD), ^{1}H nuclear magnetic resonance (^{1}H NMR), scanning electron microscopy (SEM) were performed in accordance with our previously described methods [22]. Thermogravimetric analysis (TGA) was conducted on a thermogravimetric analyzer (TG209F1 Iris, NETZSCH, Germany) with an uncovered polytetrafluoroethylene crucible under nitrogen. Two-dimensional (2D) ROESY spectra of HP-β-CD/RLZ inclusion complex were obtained in D_2O using a Bruker AVANCE II-600 NMR spectrometer (Germany) with a mixing time of 300 ms and relaxation delay of 2 s. Solid-state NMR spectroscopy (ssNMR) experiments were performed on a Bruker AV II-500 MHz NMR spectrometer using a double-tuned CP/MAS probe equipped for 4 mm (od) rotors at ambient temperature. High resolution solid-state ^{13}C CP/MAS spectra were recorded using a spectral width of 37.9 kHz, a recycle delay of 3 s, 1024 scans, and an acquisition time of 49.9 ms.

2.3.3. In vitro evaluation

Solubility studies, dissolution studies, and cytotoxicity studies were performed in accordance with our previously described methods [22]. In the dissolution studies, the samples of the pure RLZ, the HP-β-CD/RLZ physical mixtures, and the HP-β-CD/RLZ inclusion complex contained 20 mg of RLZ, and the experiments were repeated three times.

Absorption spectra were recorded with various concentrations of HP-β-CD (0 to 10.0×10^{-3} M) added to a constant RLZ concentration (2.0×10^{-5} M). Fluorescence spectra were obtained using a Cary Eclipse fluorescence spectrophotometer (Varian, USA) equipped with a 1.0 cm quartz cell with wavelengths ranging from 300 nm to 500 nm at different temperatures (25 °C, 31 °C, and 37 °C) and an excitation wavelength of 280 nm. Slit widths