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Characterization and evaluation of synthetic riluzole with β-cyclodextrin and 2,6-di-*O*-methyl-β-cyclodextrin inclusion complexes

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Riluzole (PubChem CID: 5070)
β-Cyclodextrin (PubChem CID: 444041)
2,6-Di-*O*-methyl-β-cyclodextrin (PubChem CID: 10171019)
Dimethyl sulfoxide (PubChem CID: 679)
Ethanol (PubChem CID: 702)
Methanol (PubChem CID: 887)

ABSTRACT

β-Cyclodextrin (β-CD) and 2,6-di-*O*-methyl-β-cyclodextrin (DM-β-CD) inclusion complexes with riluzole (RLZ) were prepared to improve water solubility and broaden potential pharmaceutical applications. CDs/RLZ inclusion complexes were confirmed via phase solubility studies, FT-IR spectroscopy, PXRD, DSC, ^{1}H NMR, and SEM. Phase solubility studies indicated that β-CD and DM-β-CD can form 1:1 inclusion complexes with RLZ, and the stability constants were 663.17 and 1609.07 M^{-1}, respectively. Water solubility and dissolution rate of RLZ were significantly improved in complex forms, implying that the inclusion complexes may develop pharmaceutical applications. Preliminary in vitro cytotoxicity assay also showed that RLZ hepatotoxicity was not increased in the inclusion complexes.

1. Introduction

Riluzole (RLZ, Fig. 1A) is a sodium channel-blocking benzothiazole anticonvulsant drug. In 1995, RLZ has been approved by the Food and Drug Administration for the treatment of patients with amyotrophic lateral sclerosis, a progressive neurodegenerative disorder characterized by motor neuron and corticospinal tract degeneration (Bensimon, Lacomblez, & Meininger, 1994; Lacomblez, Bensimon, Meininger, Leigh, & Guillet, 1996). RLZ has improved the treatment outcome of spinal cord injury in preclinical studies (Schwartz & Fehlings, 2001, 2002). Given that RLZ can stabilize sodium channels and inhibit glutamate release, this medication exhibits anticonvulsant and calming effects, sedative properties, and strong neuroprotection (Doble, 1996; Pratt et al., 1992).

However, RLZ is mainly administered orally because poor water solubility restrains the absorption of this drug, thereby limiting its application in dosage forms (Bruno et al., 1997). So far, many formulation approaches, such as salt formation (Tao, Zhao, Wu, & Zhou, 2009), amorphous solid dispersions (Marsac, Li, & Taylor, 2009), particle size reduction (e.g., drug nanocrystals or nanosuspensions) (Gao, Zhang, & Chen, 2008; Ghosh, Bose, Vippagunta, & Harmon, 2011), surfactants (Patel & Sarai, 2014), prodrugs (Stella & Nti-Addae, 2007), and complexation (e.g., cyclodextrins and) (Di Cagno, Terndrup Nielsen, Lambertsen Larsen, Kuntsche, & Bauer-Brandl, 2014; Huang et al., 2014), have been developed to enhance the solubility and stability of hydrophobic drugs.

Cyclodextrins are nontoxic cyclic oligosaccharides derived from starch and contain six (α-CD), seven (β-CD), eight (γ-CD), or more (α-1,4)-linked α-D-glucopyranose units. Cyclodextrins exhibit a hydrophilic outer surface and lipophilic central cavity; cyclodextrins can function as hosts for both polar and nonpolar guests, including small molecules and polymers (Davis & Brewster, 2004).

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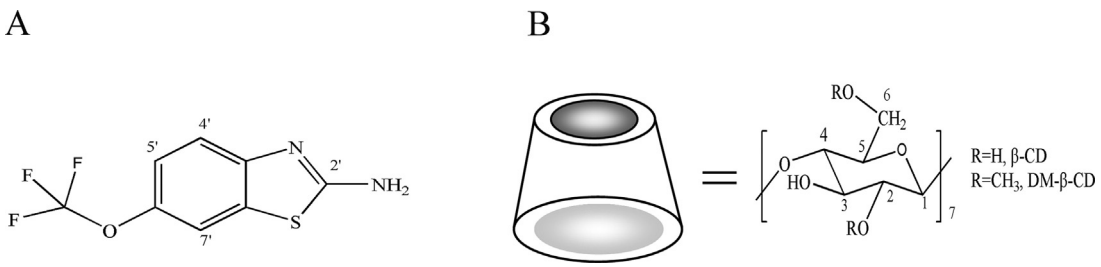

Fig. 1. Chemical structures of (A) RLZ and (B) CDs showing carbon and proton numbering in ^{1}H NMR spectra.

Cyclodextrin derivatives and polymers have also elicited increasing interest because of their higher aqueous solubility than that of native cyclodextrins, thereby potentially enhancing binding affinities and selectivity (Ciobanu et al., 2013). Complexation has been widely used with cyclodextrins as encapsulating agents to enhance solubility, stability, release, and bioavailability of drug molecules (Loftsson & Duchene, 2007; Uekama, Hirayama, & Irie, 1998). For example, conjugate cyclodextrins of ferrocenyl hydrazones exhibited excellent water solubility, improved hydrolytic stability, and significant antibacterial activity for tuberculosis (Dandawate, Vemuri, Khan, Sritharan, & Padhye, 2014). Complexes of 1-indanone thiosemicarbazones with cyclodextrin derivatives showed high water solubility and significant antiviral activity against hepatitis C virus (Glisoni et al., 2012). Consequently, studies on inclusion of different drugs in pharmaceutical applications become one of the most extensively developing areas of research (Loftsson & Brewster, 2012; Singh & Aboul-Enein, 2005). Complexation with cyclodextrins and their deriavatives has also widely used in food, agriculture and cosmetics (Loukas, Jayasekera, & Gregoriadis, 1995; Loukas, Vraka, & Gregoriadis, 1996; Zhang, Lerner, Rustrum, & Hofmann, 1999).

In the present study, complexes of RLZ were prepared using β-cyclodextrin (β-CD) and 2,6-di-*O*-methyl-β-cyclodextrin (DM-β-CD) (Fig. 1B) to improve solubility and to determine the effect of complexation on their cytotoxicity. The obtained complexes provided a highly rational use of RLZ by improving its water solubility. To explore host–guest interaction, the complexes were characterized via phase solubility diagram, Fourier transform infrared spectroscopy (FT-IR), powder X-ray diffraction spectroscopy (PXRD), differential scanning calorimetry (DSC), nuclear magnetic resonance (^{1}H NMR) spectroscopy, and scanning electron microscopy (SEM). The effects of the complexes on solubility, dissolution rate, and hepatotoxicity were also evaluated.

2. Materials and methods

2.1. Materials

RLZ (F_W = 234.20, purity ≥98%) was obtained from Yuancheng Saichuang Technology Co., Ltd. (Hubei, China). β-CD (F_W = 1134.98, purity ≥99%) was purchased from Kelong Chemical Co., Ltd. (Chengdu, China). DM-β-CD (F_W = 1331.39, purity ≥98%) was purchased from Best Reagent Co., Ltd. (Chengdu, China). 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) and dimethyl sulfoxide (DMSO) were purchased from Sigma (Sigma-Aldrich, Inc.). Other reagents and chemicals were of analytical grade and used without further purification. Triple-distilled water was used throughout the experiment.

2.2. Preparation of inclusion complexes

RLZ inclusion complexes with CDs (β-CD and DM-β-CD) were prepared in molar ratio of 1:1 via co-precipitation and vacuum-drying techniques, respectively. Co-precipitation was performed as described by Marcolino, Zanin, Durrant, Benassi, and Matioli (2011) with few modifications. β-CD (3.40 g) was completely dissolved in 30 mL of triple-distilled water and stirred at 70 °C. RLZ (0.70 g) was dissolved in ethanol at 70 °C and added dropwise to the solution. The mixture was then magnetically stirred for 4 h. The final solution was filtered, and the filtrate was cooled overnight at 1 °C. Subsequently, the solid residue was washed thrice with ethanol and triple-distilled water to remove unreacted RLZ and β-CD. The final solid inclusion complex was collected after drying at 70 °C.

DM-β-CD (1.33 g) and RLZ (0.23 g) were completely dissolved in 10 mL of methanol. After magnetic stirring at 40 °C for 4 h, the resulting solution was filtered and vacuum dried at 40 °C to collect the final inclusion complex.

The physical mixture was prepared by simply mixing RLZ and CDs with 1:1 molar ratio on a vortex mixer for 5 min to obtain a homogeneous blend.

2.3. Phase solubility studies

Phase solubility studies were performed as described by Higuchi and Connors (Higuchi & Connors, 1965). An excess amount of RLZ (25 and 45 mg for β-CD and DM-β-CD assays, respectively) was added to aqueous solutions (10 mL) containing different concentrations of β-CD (0 mM to 16 mM) and DM-β-CD (0 mM to 30 mM). The flasks were sealed and heated in an autoclave (110 °C for 30 min), and allowed to cool down to room temperature. Then the flasks were shaken for 7 d (Preliminary experiments showed that 7 days are more than enough time to reach solubility equilibrium.) at 25 °C. Afterwards, the suspensions were filtered through a 0.45 μm Millipore filter, and the RLZ concentration in the filtrate was properly diluted and analyzed at 261 nm using a UV–vis spectrophotometer (TU-1901, Purkinje General Instrument, China).

2.4. Inclusion complex characterization

FT-IR spectra were recorded on a Nicolet 6700 spectrometer (Thermo Fisher Scientific, Waltham, USA) based on KBr disk technique. KBr disks were prepared with 1 mg of samples in 100 mg of KBr. FT-IR spectra were measured in the scanning range of 4000 cm^{-1} to 400 cm^{-1} at ambient temperature.

PXRD patterns were obtained at 25 °C by using X'Pert PRO (PANalytical, Almelo, Netherlands) diffractometer and Cu $K\alpha 1$ radiation over the angular range of 5° to 50°, 2θ with step size of 0.01313°, and counting time of 30 ms/step.

DSC analyses were performed by comparing the thermal behaviors of RLZ, CDs, and their physical mixtures and inclusion complexes. Samples (5 mg each) were placed in sealed aluminum pans, and the experiments were run in DSC Q200 (TA Instruments Co., New Castle, DE, USA) at 10 °C/min heating rate over a wide temperature range (30 °C to 300 °C) under a nitrogen atmosphere.