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Alleviating the hepatotoxicity of trazodone via supramolecular encapsulation

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ABSTRACT

In order to develop a novel strategy to alleviate the inherent hepatotoxicity of antidepressant trazodone (TZ), Cucurbit[7]uril (CB[7]) was adopted as pharmaceutical excipients and was studied for its capability to reduce the hepatotoxicity of TZ via supramolecular encapsulation. CB[7] was found to form strong 1:1 host-guest complexes with TZ and its metabolite *m*-chlorophenyl piperazine (mCPP), with binding constants of 1.50 (± 0.13) × 10^6 M^{-1} and 6.90 (± 0.49) × 10^5 M^{-1}, respectively. The supramolecular complexations were examined by ^{1}H NMR and UV-visible spectroscopic titrations, ESI-MS and ITC. In the presence of 0.5 mM CB[7], the IC_{50} values of TZ and mCPP on a human normal liver cell line L02 were increased from 215.5 ± 3.3 μM to 544.1 ± 51.2 μM, and from 166.8 ± 3.8 μM to 241.7 ± 6.8 μM, respectively. Evaluation on a zebrafish model demonstrated that CB[7] (0.1 mM) significantly alleviated the TZ induced liver toxicity, as shown by the level of liver degeneration, liver size and yolk sac retention. Our study may provide a supramolecular strategy to alleviate the hepatotoxicity induced by TZ and its metabolite mCPP, and this strategy may be extendable to other drugs that have inherent hepatotoxicity or other adverse effects.

1. Introduction

Trazodone (TZ) (Fig. 1) is a FDA-approved drug that was developed for the treatment of depression, as its active metabolite, *m*-chlorophenyl piperazine (mCPP) (Fig. 1), acting as a serotonin receptor agonist-antagonist, could selectively regulate the reuptake of serotonin in the brain(Brogden et al., 1981). However, some patients often suffer adverse effects during the administration of TZ, among which liver injury has come to the fore. For instance, it has been reported that TZ could cause hepatitis even after short-term administration of a relatively large dose(Brogden et al., 1981; Haria et al., 1994). TZ-induced hepatoxicity mainly involves two mechanisms, namely oxidative stress and intracellular organelles dysfunction(Taziki et al., 2013; Najibi et al., 2016). Recently, it has been demonstrated that the hepatotoxicity of TZ might be attributed to its active metabolite mCPP(Wen et al., 2008). Current strategies for the hepatotoxicity management are focused on combining other hepatoprotective drugs into the co-administration. In particular, taurine and melatonin are often employed as combination medicines with TZ to alleviate TZ-induced hepatotoxicity, as these two compounds were found to show hepatoprotective effect(Taziki et al., 2013). However, this strategy would have to force patients to take these additional drugs that may cause allergic reactions or other adverse events, particularly patients often take antidepressant for a long period of time. Therefore, it is highly desirable to develop alternative strategies for the hepatotoxicity alleviation of TZ, e.g. through pharmaceutical formulation.

Cucurbit[*n*]uril (CB[*n*], *n* = 5–8, 10, 13–15) are novel macrocyclic host molecules comprising *n* glycoluril monomers linked by 2*n* methylene bridges. These pumpkin-shaped molecules possess one hydrophobic cavity and two identical hydrophilic portals fringed by carbonyl groups, which may selectively encapsulate a variety of guest molecules (Isaacs, 2009; Masson et al., 2012; Barrow et al., 2015). In particular, CB[7] (Fig. 1) has drawn the most attention as a potential drug delivery carrier aiming at improving therapeutic efficacy, and/or alleviating adverse effects(Kuok et al., 2017), as CB[7] has relatively good aqueous solubility(Macartney, 2011; Walker et al., 2011; Shetty et al., 2015), well-studied biocompatibility(Chen et al., 2015a; Li et al., 2017a), and appropriate size that can encapsulate a variety of biomedically relevant molecules with higher binding affinities than those of CDs(Macartney, 2011; Shetty et al., 2015). During the past several years, significant efforts have been devoted by our group to investigating CB[7]'s capability to regulate the physicochemical and biological properties of the guest drug molecules(Li et al., 2015; Li et al., 2016a; Li et al., 2016b; Kuok et al., 2017; Li et al., 2017b; Yang et al., 2017a; Yin and Wang, 2017). For instance, we have successfully demonstrated alleviation of the cardiotoxicity of sorafenib and clofazimine by supramolecular

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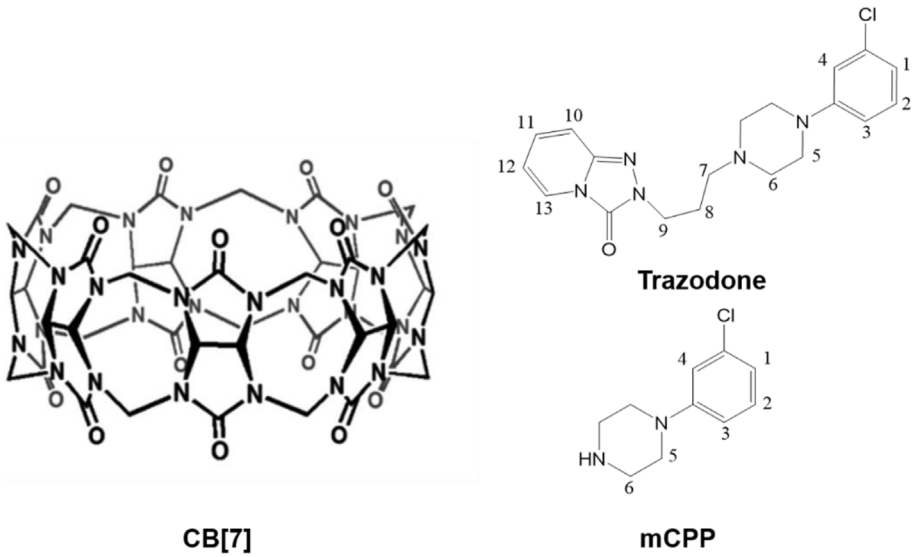

Fig. 1. Molecular structures of CB[7], Trazodone (TZ) and mCPP. The hydrogens in TZ and mCPP are numerically labeled for ^{1}H NMR assignment.

encapsulation of CB[7](Li et al., 2016c; Yang et al., 2017b). Inspired by these preliminary success, we decided to investigate the supramolecular encapsulation of TZ and its metabolite mCPP by CB[7] and to evaluate the hepatotoxicity of trazodone in the absence and in the presence of CB [7] with both *in vitro* and *in vivo* zebrafish models. This work will likely provide a new strategy in reducing the adverse effects of TZ via a facile supramolecular encapsulation, and further promote the scope of application of CB[7] as a novel pharmaceutical excipient.

2. Experimental

2.1. Ethics statement

All animal experiments were performed in accordance with the ethical guidelines of the Institute of Chinese Medical Sciences, University of Macau, and the protocols were approved by the Animal Ethics Committee at the University of Macau.

2.2. Materials and instrumentation

The host molecule CB[7] was synthesized and purified by using a reported method(Day et al., 2001). Trazodone (TZ) hydrochloride and 1-(3-chlorophenyl) piperazine (mCPP) were purchased from J&K chemical (Shanghai, China) and ARK Pharm Inc (USA), respectively. MTT (3-(4,5-dimethylthiazol-2-yl)-2,5- diphenyl tetrazolium bromide) was purchased from Sigma Aldrich (St. Louis, MO, USA). RPMI culture solution, penicillin-streptomycin (PS), trypsin, phosphate buffered saline (PBS) and fetal bovine serum (FBS) were purchased from Gibco (Carlsbad, CA, USA). The E3 medium was composed of 5 mM NaCl, 0.17 mM KCl, 0.33 mM $CaCl_2$, and 0.33 mM $MgSO_4$ (pH 7.2–7.3).

The ^{1}H NMR spectra were acquired on a Bruker Ultra Shield 600 PLUS NMR spectrometer. The ESI-MS spectrometry analysis was conducted using a Thermo LTQ OrbiTrap XL instrument equipped with an ESI/APcI multiprobe. The UV-visible titration was performed using a HACH DR6000 UV-visible spectrometer with a 1.0 cm path length quartz cell. The isothermal titration calorimetry (ITC) test was conducted on a Malvern MicroCal PEAQ-ITC and the results were analyzed using a built-in software. The MTT assay was monitored by microplate reader (Spectra Max M5 Microplate Reader, Molecular Devices, USA). The morphology of zebrafish was observed via microscope (Olympus, SZ61). The Milli-Q water was used throughout the whole study.

2.3. UV-visible spectroscopic titration

Binding stoichiometry For the Job plot titration, the principle is that the sum concentration of the host and guest needs to be kept as a constant. Therefore, the guest molecules (TZ 0.18 mM and mCPP 0.08 mM) and the host (CB[7] 0.18 mM for TZ, 0.08 mM for mCPP) solutions were prepared as stock solutions. During the titration, the solutions were prepared by simple mixing different volumes of the prepared host and guest stock solutions (total 3 mL) with the ratio [CB [7]]/([Guest] + [CB[7]]) adjusted from 0 to 1.0 by a step of 0.1.

2.4. ITC experiment

TZ (0.1 mM), mCPP (0.1 mM) and CB[7] (1 mM) solutions were individually prepared via diluting the corresponding stock solution with degassed aqueous solution (for mCPP titration, the pH of both mCPP and CB[7] was adjusted to 2.4). A solution (0.2 mL) containing TZ (or mCPP) was added to the sample cell, while another solution (0.04 mL) containing CB[7] was placed to syringe for titration. The ITC titration was conducted by titrating 19 drops (0.4 μL for the first drop and 2 μL per drop for 18 drops) of CB[7] solution at T = 25.0 °C, and the heat evolution was recorded. The heat generated from the dilution of CB[7] was corrected by titrating ultrapure water (or pH = 2.4 solution for the case of mCPP) with CB[7] and the data was subtracted from those of the host-guest titrations. Computer simulations (curve fitting) and data analysis were performed using the built-in software of MicroCal PEAQ ITC Analysis Software 1.1.0.1262.

2.5. Cytotoxicity assay (MTT test)

Cell lines and Culture L02, a human normal liver cell line, obtained from the Committee of Type Culture Collection of the Chinese Academy of Sciences (Shanghai, China), was cultured in DMEM medium supplemented with 10% FBS and 1% penicillin-streptomycin (PS) in a humidified environment at 37 °C with 5% CO_2. The medium was changed every 2 days.

To evaluate the liver toxicity of TZ and mCPP in the absence and presence of CB[7], L02 cell line was tested by colorimetric 3-(4, 5-dimethylthiazol-2-yl) 2, 5-diphenyltetrazolium bromide (MTT) assay. The standard treatments for L02 cells were as follows: the cells were used until they reached 70%–80% confluence and seeded onto 96-well plates (4000–5000 per well) to allow to attach overnight. The test compounds were added with final doses ranging from 100 to 300 μM TZ or mCPP (in the absence and in the presence of 500 μM CB[7]), and the cells