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A strategy to improve the oral availability of baicalein: The baicalein-theophylline cocrystal

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ABSTRACT

Baicalein (BE) is a flavonoid compound derived from the roots of Scutellaria baicalensis. It has widely been used as anti-oxidant, anti-virus, anti-bacteria, anti-inflammatory and anti-allergic therapies. Due to its poor water solubility (16.82 μg/ml), the therapeutic effectiveness and oral bioavailability of Baicalein are highly limited. The purpose of this study was to investigate the possibility of baicalein-theophylline (BE-TH) cocrystals to achieve the simultaneous enhancement in dissolution and oral bioavailability of BE. The cocrystal had the new characteristic of scanning electron microscopy, differential scanning calorimetry thermograms and X-ray powder diffraction. Compared with coarse powder of BE, BE-TH cocrystals had significantly improved the solubility of BE. The dissolution test showed that the BE-TH cocrystals demonstrated 2.2-fold and 7.1-fold higher rate of dissolution than that of BE coarse powder in HCl (pH = 1.2) and phosphate buffer (PBS, pH = 6.8), respectively. Moreover, the cocrystals exhibited a 5.86-fold higher area under the curve in rats after the oral administration. This investigation enriched the present understanding of cocrystals on their behavior in vitro and in vivo, and built the groundwork for future development of BE as a promising compound into efficacious drug products.

1. Introduction

Scutellaria baicalensis Georgi (Huang Qin), an East Asian skullcap plant that contains abundant flavonoids, is widely used in traditional Chinese medicine as a remedy for the clinical treatment of hyperlipemia, hypertension, atherosclerosis, dysentery, common cold and inflammatory and tumor [1–3]. Baicalein (5, 6, 7- trihydroxyflavone, BE), a natural bioactive phenolic flavonoid compound, is primarily available from the root of the Huang Qin [4]. The pharmacological effects of BE include against oxidants, free radicals, cancer, sclerosis, bacteria, inflammation, as well as being cardio- and neuroprotective [5–7]. However, the innate drawbacks of BE are its extremely low aqueous solubility and poor oral absorption, which greatly limited its therapeutic applications.

Among the methods for improving the solubility of bioactive compounds, pharmaceutical cocrystals offer a great potential of overcoming the problem associated with solubility. A cocrystal is a multi-component crystalline complex which consists of two or more solid components (at ambient conditions) in a definite stoichiometric ratio held together via noncovalent interactions [8, 9]. The pharmaceutical cocrystal is a new compound which can incorporate pharmaceutically acceptable ligands and drug into the same crystal lattice. Nowadays, many cocrystals have already been produced and achieved satisfactory results. Liu et al. [10] prepared the cocrystal of myricetin with proline by solution crystallization. The results showed that the dissolution rate of myricetin in the cocrystal was 7.69 times higher than that of coarse myricetin. The oral bioavailability of the cocrystal was approximately 3.03 times higher than that of myricetin. Du et al. [11] prepared two cocrystals of lamotrigine formed with 4, 4′-bipyridine and 2, 2′-bipyridine successfully, and both cocrystals exhibited the improved dissolution rate and solubility. The dexlansoprazole-isonicotinamide cocrystals also profiled a very apparent increase in solubility compared with the pure drug [12]. Thus pharmaceutical cocrystals are believed as the promising approach to improve the solubility even the bioavailability of BE.

BE possesses many competitive hydrogen bonding sites in its chemical structure, i.e. donors and acceptors (3 hydroxyls and 1 carbonyl), providing the possibility for the formation of cocrystals with the proper

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coformers. Zhu et al. [13] reported four cocrystals of BE with enhanced solubility and increased bioavailability. More specifically, baicalein-nicotinamide cocrystal formulation could significantly enhance the oral bioavailability than that of crystalline BE [14, 15].

Theophylline (TH) is known to have good possibilities for cocrystals formation. The molecular structure of TH contains hydrogen-bond acceptors and donors, so that it can form cocrystals with hydrogen bonds on the basis of suitable ligands. TH was often used as the main drug in the preparation of cocrystals [16]. For example, four theophylline cocrystals formed with oxalic acid, malonic acid, maleic acid and glutaric acid were prepared to illustrate the enhanced physical stability of TH via cocrystallization [17]. Most researchers only used TH as the main drug to study the molecular structure of cocrystals, according to previous reports, but did not use its ligand-dominant structure to examine whether it could act as a ligand to increase the dissolution rate and the bioavailability of the poorly soluble natural products such as BE. Therefore, it seems critical to evaluate TH as a commonly used and readily available coformer to form some novel pharmaceutical cocrystals with natural products.

Coincidentally, BE-TH cocrystals were prepared and investigated in the previous experiment [13], but no more solubility tests or in-vivo pharmacokinetics were held for further evaluation. Therefore, it was meaningful to investigate whether BE-TH cocrystals could exhibit some unique advantages in vitro (solubility) and in vivo (bioavailability) when it is used as a ligand migrated to the hot-drug BE.

In this work, BE was selected as the typical poorly soluble drug model, pharmaceutical cocrystals of BE-TH were prepared using solution crystallization method, and the cocrystals were characterized by scanning electron microscopy, differential scanning calorimetry thermograms and X-ray powder diffraction. Moreover, the in vitro and in vivo evaluations of cocrystals were also investigated to assess the potential of cocrystals for poorly soluble drugs. We believed firmly that the formation of cocrystals is a promising strategy to overcome the obstacles exist in the field of natural products such as BE.

2. Methods and materials

2.1. Materials

Baicalein (BE, > 98% purity) was purchased from Nanjing Zelang Medical Technology Co. Ltd. (Nanjing, China). Theophylline (TH, > 98.5% purity) was purchased from J&K Scientific Ltd. (Beijing, China). BE (98.5% purity, batch number: 111595–201306), baicalin (BA, 93.5% purity, batch number: 110715–201619) and carbamazepine (CBZ, 98.5% purity, batch number: 110723–201413) as internal standard (IS) were kindly gifted by National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Methanol of HPLC grade was gained from Fisher Scientific Co. (Fair Lawn, New Jersey, USA). All agents were analytical grade and purified Milli-Q water (Millipore, Billerica, MA, USA) was used throughout all experiments.

Sprague-Dawley rats (weighting 250 ± 20 g; Shanchuanhong Experimental Animal Tech Co. Ltd.; Tianjin, China; Licence: SCXK 2014–0001, Tianjin, China) were housed at room temperature with free access to drinking water but fasted overnight before the administration of drugs. The protocol and any amendments or procedures involving the care or use of animals in this study were in accordance with the regulations for animal experimentation issued by the State Committee of Science and Technology of China and approved by TJUTCM's Institutional Animal Care and Use Committee (Document number TCMLAEC 20170020).

2.2. Preparation of BE-TH cocrystals

BE and TH were used to prepare BE-TH cocrystals according to a certain proportion. The method of preparation of BE-TH cocrystals at a molar ratio of 1: 1 was as follows: BE (540 mg) was dissolved in 80 ml of acetone, stirred and ultrasonicated until BE was completely dissolved in acetone, and then 360 mg TH was added to the above solution, stirred and ultrasonic to TH no longer dissolve, and the rotor was placed on a magnetic stirrer at 200 rpm for 15 h, standing for 10 h, and then filtered by filter membrane with the 0.45 μm size), the acetone in the sample was evaporated and then BE-TH cocrystals were harvested.

Table 1
Mass parameters for the assay of BA, BE and CBZ in rats plasma.

Compound	Mode	Ionization	Fragmentor (V)	MS1 → MS2	Collision Energy (eV)
CBZ (IS)	MRM	ESI^+	140	237.1 → 193.8	18
BA	MRM	ESI^+	100	447.09 → 270.8	38
BE	MRM	ESI^+	160	271.06 → 122.9	14

2.3. Differential scanning calorimetry (DSC)

DSC analysis was carried out using differential scanning calorimeter (Jade DSC, PekinElmer Inc. Waltham, MA, USA). About 5 mg of each sample (BE coarse powder, TH coarse powder, physical mixture and BE-TH cocrystals) was accurately weighed and placed in an aluminium pan, and an empty pan was used as a reference. The study was carried out between ranges of 30 °C to 350 °C, at a scanning rate of 10 °C/min.

2.4. Powder X-ray diffraction (PXRD)

PXRD patterns of BE coarse powder, TH coarse powder, physical mixture and their cocrystal samples were recorded using a Ni filtered Cu Kα radiation source (D/MAX-2550 V, Rigaku Co., Japan). The generator was set to 40 kV and 100 mA and the radiation wavelength was 1.542 Å. The samples were continuously scanned from 5° to 80° (two-theta) at a scanning rate of 5°/min. The data were analyzed by MDI Jade 6.0 software.

2.5. Scanning electron microscopy (SEM)

The surface morphology of BE coarse powder and BE-TH cocrystals was evaluated by SEM (JSM-7500F, JEOL Ltd., Japan) at a stepped-up voltage of 10.0 kV. The samples were covered with gold on a holder and desiccated in a vacuum and observed at different magnifications.

2.6. In vitro dissolution test

To evaluate the enhanced dissolution rate of BE-TH cocrystals, in vitro dissolution tests were executed using basket method with dissolution testing apparatus (DT-820, ERWEKA Co., Germany). 0.1 M HCl and PBS (pH 6.8) both contained 0.2% SDS-Na were employed as the dissolution medium. BE coarse powder, physical mixture and BE-TH cocrystals (contained 18 mg BE each) were put into the hard capsules and then immersed into 900 ml dissolution media. The temperature and stirring rate were maintained at (37 ± 0.5) °C and 100 rpm, respectively. Two ml of aliquots were withdrawn at 5, 10, 15, 30, 45, 60, 90, 120, 180, 240, 300, 360 min, and meantime 2 ml of fresh dissolution medium were added. The samples were assayed by HPLC (Agilent 1200, CA, USA) after filtration with 0.45 μm microporous membrane. The test was executed with three replicates for each group, and the data were reported as a mean ± SD. A plot of the cumulative dissolution of BE against time was constructed to illustrate the drug dissolution profiles [18]. Cocrystals with elevated dissolution rates could be expected as optimistic absorption in pharmacokinetics.