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The pharmacokinetics and anti-inflammatory effects of chelerythrine solid dispersions *in vivo*

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ABSTRACT

Chelerythrine (CHE) is a quaternary benzo [c] phenanthridine alkaloid which has a wide array of pharmacological properties. The purpose of this study was to prepare CHE solid dispersions (SDs), which was aimed at increasing bioavailability and anti-inflammation of CHE. Firstly, CHE-SDs was prepared with different carriers using solvent evaporation method. Then the dissolution study in vitro showed that the optimal carrier was PVP K30 and the best proportion of CHE and PVP K30 was 1:10. Solid state characterization was evaluated by the approach of differential scanning calorimetry (DSC). DSC analysis indicated the complete transformation of CHE in the SDs from crystalline to amorphous state. Finally, the pharmacokinetic study demonstrated the relative bioavailability of CHE in CHE-SDs was significantly improved in comparison to that of CHE solution. AUC of CHE-SDs was about 2.36-fold higher than that obtained with CHE solution. Peak concentration (C_{max}) and elimination half-life ($t_{1/2,\beta}$) were both higher for CHE-SDs than that for CHE solution. Conversely, total body clearance (CL_s) and apparent volume of distribution ($V_{f(c)}$) were lower for CHE-SDs in comparison with the CHE solution. CHE-SDs significantly improved the anti-inflammatory effect of CHE through inhibiting the levels of TNF-α, IL-6 and NO in mice serum. In summary, the bioavailability and anti-inflammation activity of CHE were significantly improved by making into CHE-SDs.

1. Introduction

A quatemary benzo [*c*] phenanthridine alkaloid chelerythrine (CHE) (Fig. 1) is widely distributed in the *Papaveraceae* and *Rutaceae* families of plants, which is often used as a traditional Chinese medicine, officially listed in the Chinese Pharmacopoeia [1]. It has displayed a wide array of biological activities, among which antimicrobial, antifungal, antineoplastic and anti-inflammatory activities are the most important for practical applications [2–4]. It also exhibits a broad range of cytotoxic activity in vitro and antitumor activity in tumor cells [5]. However, the research of this compound is hampered by its poor water solubility. A promising strategy to overcome this disadvantage involves the development of suitable drug carrier systems.

Solid dispersions (SDs) is one of the most effective approaches to improve the solubility and dissolution rate and hence the bioavailability of drugs [5,6]. Solubility properties are one of the main physicochemical parameters in new drug candidates and the drug might be dispersed as molecularly or dispersed as small particles in a solid dispersion, which aim to improve the oral drug oral absorption and bioavailability at a safe and pharmacologically neutral carrier matrix. On the other hand, many materials can choose to preparation SDs [7,8]. SDs is mixtures of the drug and suitable carrier polymers. Polymers such as Polyvinyl Pyrrolidone (PVP), Polyethylene Glycols (PEGs), Poloxamer, Polymethacrylate, or natural polymers can be used to obtain SDs [9]. In particular, PVP K30 has been extensively studied as an efficient carrier in SDs of various drugs and as a result of its ability to retard and inhibit recrystallization of drugs [10]. PEGs are widely used as vehicles for SDs because of their low melting point, rapid solidification rate, capability of forming solid drug solutions, low toxic and low costs [11]. Poloxamers are intensely concerned owing to its high biocompatibility and low toxicity [12]. Making the amorphous SDs is one of the effective methods to increase the dissolution rate thereby improving the bioavailability of drugs [13]. The dispersion

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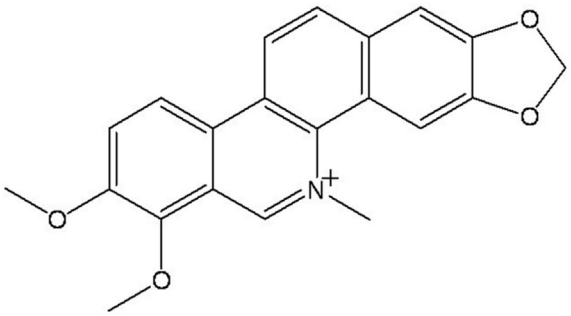

Fig. 1. Structure of CHE.

of the drug molecules into the polymer matrix is another factor which increases the dissolution rate of the drug [14]. Recently, some useful methods to prepare SDs include hot-melt extrusion [15], spray-drying [16], rotary evaporation [17], freeze-drying [18], solvent evaporation and mechanical milling [19] and so on. The method of solvent evaporation involves the dissolution of the drug and polymer in a solvent or a mixture of solvents such as ethanol, dichloromethane and chloroform, which is then evaporated. There are many approaches to identify SDs such as X-ray power diffraction, differential scanning calorimetry (DSC), infrared (IR), Raman spectroscopy, polarized light microscopy and in vitro dissolution testing. Recently, some studies have indicated that the formation of SDs can improve the oral bioavailability of drugs [20,21].

The aim of this article is to produce CHE-SDs and improve bioavailability of CHE. In this study, we successfully prepared CHE-SDs. Moreover; we evaluated bioavailability of CHE-SDs through the pharmacokinetic experiment and measured its anti-inflammatory activity in LPS-induced endotoxic shock experiment after single dose administration - of CHE-SDs in comparison with CHE solution.

2. Materials and methods

2.1. Reagents

CHE was purchased from Xi'an Honson Biotechnology Co. Ltd. and identified by the Pharmacognosy Laboratory, School of Pharmacy, Xi'an Jiaotong University (Xi'an, China). Berberine (99% pure) as internal standard (IS), was purchased from National Institute for the Control of Pharmaceutical and Biological Products (Xi'an, China). PVP K30 was supplied by BASF (Germany). PEG 6000 was provided by Tianjin Kermel Chemical reagent Co. Ltd. Lutrol F127 was obtained from Beijing Fengli Jingqiu Commerce and Trade Co. Ltd. Methanol was High pressure liquid chromatography (HPLC) quality grade solvent and was purchased from Tianjin Kermel Chemical reagent Co. Ltd. The enzyme-linked immunosorbent assay (ELISA) kit for mouse interleukin 6 (IL-6) and tumor necrosis factor alpha (TNF-α) were purchased from R&D Systems (Minneapolis, MN, USA). Lipopolysaccharide (LPS) and Griess reagent were purchased from Sigma (St. Louis, MO, USA). All other reagents used in the study were of analytical grade.

2.2. Animals

Sprague-Dawley rats (220–250 g) and Male Kunming mice (22–25 g) were purchased from Laboratory Animal Center, Xi'an Jiaotong University (Xi'an, China). Animals were kept on a 12 h light/dark circle and were fed with standard pallet diet and water *ad libitum*. All the animals were acclimatized to the laboratory conditions for a week prior to the experiments. The rats were fasted for 12 h before drug administration, although water was available. All experimental procedures utilizing mice were in accordance with National Institute of Health guidelines.

2.3. Preparation of solid dispersions and physical mixture

SDs were prepared by solvent evaporation method using ethanol as solvent containing amount of CHE and PEG6000, PVP K30 or Lutrol F127 at a ratios of 1:3, respectively [22]. Then the mixtures were added to the ethanol and dissolved with magnetic stirring for 24 h. The mixed solution was made dry naturally at room temperature. CHE was mixed with amounts of PVP K30 (ratio 1:3, 1:5, 1:7 and 1:10), followed by the addition of ethanol, then they were made SDs by the same method as above. Physical mixtures were prepared by gently mixing appropriate quantities of CHE and PVP K30 in a mortar.

2.4. Dissolution study

2.4.1. HPLC analysis

2.4.1.1. HPLC system. The HPLC analysis was carried out using a Shimadzu LC-2010A HPLC system (Japan) which consisted of a SPD-10AVP UV-detector and a CLASS-VP chromatographic working station. The separation was performed on a kromasil 5u-C_{18} column (150 mm × 4.6 mm, 5 μm). The mobile phase was acetonitrile-0.1% phosphoric acid in water (30/70, v/v). It was freshly prepared daily and filtered through 0.45 μm membrane filter. The flow rate was kept constant at 1.0 mL/min and the temperature of oven was maintained at 37 °C. The samples were detected at 272 nm. The injection volume for all the samples was 20 μL. The methods were validated in terms of linearity, specificity, precision, reproducibility, stability and recovery.

2.4.1.2. Standard and working solutions. CHE stock solution was prepared by dissolving 5.0 mg of CHE in 10 mL of methanol. Working solutions were prepared from the stock solution by dilution in methanol. All stock solutions were stored at 4 °C but equilibrated to room temperature before use.

2.4.1.3. Linearity. The standard curve was established through linear regression of the peak area and the reference solution at different concentrations (31.25, 62.5, 125, 250, 500 μg/mL) for CHE, described as $Y = a + bX$.

The lowest limit of detection (LLOD) was defined as the amount that could be detected at a signal-to-noise ratio of 3. The LLOD was determined in three replicates with a precision rate of less than 20%.

2.4.1.4. Specificity. PVP K30 solution, CHE reference solution, SDs solution were injected into the HPLC system for analysis, respectively. The chromatograms were recorded.

2.4.1.5. Precision. The intra-day and inter-day precision rates were analyzed by determining reference solutions at three different concentrations (31.25, 125, 500 μg/mL) over three consecutive days. The reference solutions were tested using five replicates and calculated with calibration curve. Precision was estimated as the percentage RSD.

2.4.1.6. Stability. The same sample solution was injected into the HPLC system to determine the peak area at 0, 2, 4, 6, 8, 12, 24 h, respectively. Stability was estimated as the percentage RSD.

2.4.1.7. Recovery. Three different concentrations of CHE reference solutions (31.25, 125, 500 μg/mL) were analyzed as the above HPLC