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Research paper

Enhanced oral bioavailability of 6-bromo-3-n-butylphthalide by self-nanoemulsifying system

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ABSTRACT

The objectives of this study were, first, to prepare 6-bromo-3-n-butylphthalide self-nanoemulsifying system (6-BBP-SNES); and second, to enhance the water solubility and oral bioavailability of 6-BBP. Pseudo-ternary phase diagram was constructed to identify an efficient self-emulsification region. Then, formulations were optimized by assessing the globule size and polydispersity. The composition of optimized 6-BBP-SNES was 6-BBP (10% w/w), Isopropyl myristate (40.5% w/w), Polyoxyethylene hydrogenated castor oil (36% w/w) and Absolute ethanol (13.5% w/w), with the globule size (80.50 ± 0.47 nm), polydispersity (0.150 ± 0.027) and an infinite dilution capability. In vitro drug release of SNES was above 80% within 5 min, whereas that of the suspension was less than 20%. Furthermore, the C_{max} of 30 mg/kg of SNES after oral administration was 1.57-fold higher than that of 100 mg/kg of 6-BBP suspension. Besides, the absolute bioavailability of SNES was found to be 47.55%, whereas that of the suspension was only 8.73%, which increased by approximately 5.45-fold. These results demonstrated SNES could be promising to improve the oral efficacy of 6-BBP, and had potential clinical application value.

1. Introduction

6-bromo-3-n-butylphthalide (6-BBP) was derived from l-3-n-butylphthalide (l-NBP) (The structures were shown in Fig. 1), which is the main component of the volatile oil from Wildcelery Herb and many other umbelliferae and composite plants [1]. l-NBP was approved by the State Food and Drug Administration (SFDA) of China in November 2002. It has many kinds of pharmacological activities, such as reducing blood pressure, improving the microcirculatory system of human's brain, ameliorating nervous function deficiency, antiasthma, inhibiting platelet aggregation, improving the hemodynamic characteristics, antitumorigenesis and so on [2,3]. However, l-NBP is a viscous and volatile oily liquid that is difficult to purify and preserve. Accordingly, 6-BBP was semi-synthesized, having excellent physicochemical properties, such as proper crystal at room temperature and high purity. Besides, previous study showed that 6-BBP was more active than l-NBP [4]. Furthermore, its LD_{50} was higher than that of l-NBP's on mouse acute toxicity by way of intraperitoneal injection [2]. However, 6-BBP, a white crystalline powder with high lipophilicity, is a poorly water-soluble and poorly permeable drug belonging to the class IV of the biopharmaceutical drug classification system (BCS IV), which seriously affect its oral bioavailability.

An approach, which will increase the drug solubility and improve the oral bioavailability, is highly desirable. In fact, various formulation strategies have been adopted including the use of solid dispersion [5], surfactant [6], liposomes [7], permeation enhancers [8]. These approaches have been successful in selected cases. Apart from these approaches, the self-nanoemulsifying drug delivery systems (SNEDDS), which are well known for their potential to improve the aqueous solubility and oral absorption of lipophilic drugs [9–12], are also worth considering.

SNEDDS are isotropic mixtures of oil(s), surfactant(s), and co-surfactant(s). Upon mild agitation followed by dilution in aqueous media, such as GI fluids, these systems can rapidly form oil-in-water (o/w)-type nanoemulsions of globule sizes ranging from 20 to 200 nm [13–15]. When compared with general emulsions, which are sensitive and metastable dispersed forms, SNEDDS are physically stable formulations that are easy to prepare and can be

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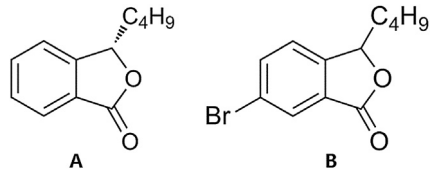

Fig. 1. Chemical structures of l-NBP (A) and 6-BBP (B).

formed within seconds. In addition, SNEDDS are characterized by high solvent capacity and small particle size. Thus, for lipophilic drug compounds that exhibit dissolution rate–limited absorption, these systems may offer an improvement in the rate and extents of absorption and result in more reproducible blood–time profiles due to the presence of the nanometer sized droplets [9]. Besides, SNEDDS can reduce the gastrointestinal side effects, enhance permeation across the intestinal membrane, and reduce or eliminate food effect [16–18]. Furthermore, SNEDDS inhibit P-glycoprotein efflux transporter, and thus enhance the bioavailability of drugs [19]. Moreover, SNEDDS can be filled directly into soft or hard gelatin capsules due to their anhydrous nature enabling its administration as a unit dosage [20]. At present, some SNEDDS formulations, such as Sandimmune® and Sandimmun Neoral® (cyclosporine A), Norvir® (ritonavir) and Fortovase® (saquinavir), have been successfully developed and applied in the pharmaceutical market [9]. Consequently, we designed 6-BBP self-nanoemulsifying system (6-BBP-SNES), which would improve the poor water solubility and low bioavailability of 6-BBP.

The purposes of this study were to prepare and characterize the optimal formulation of 6-BBP-SNES and to assess its release rate in vitro and bioavailability in beagle dogs.

2. Materials and methods

2.1. Materials

6-BBP (purity > 98%, batch No: 2013091001) was synthesized by the Department of Chemistry, Zhengzhou University (Henan, China) [2]. Polyoxy 40 hydrogenated castor oil (Cremophor RH40®) was purchased from BASF (Ludwigshafen, Germany). Isopropyl myristate (IPM) was obtained from Sinopharm Chemical Reagent Co., Ltd (Beijing, China). Absolute ethanol was provided by Tianjin Kemiou Chemical Reagent Co., Ltd (Tianjin, China). Acetonitrile and methanol of HPLC grade were obtained from Siyou Chemical Reagent Co., Ltd (Tianjin, China). All other chemicals used were of analytical grade.

2.2. Animals

Twelve healthy male beagle dogs, similar in age (2 years) and weight (10.80 ± 0.5 kg), were provided by Shenyang Kangping Institute of Laboratory Animals (Shenyang, China). The dogs were divided into three groups at random and kept under standard laboratory conditions, temperature at 25 ± 2 °C and relative humidity (55 ± 5%). They were acclimated for at least one week before experiment, and received a standard diet and water ad libitum. All animal experiments were evaluated and approved by the animal and ethics review committee of Faculty of Zhengzhou University, China.

2.3. Solubility studies

The solubility of 6-BBP in distilled water and various oils, surfactants, co-surfactants was measured using the shake flask method. An excess of 6-BBP powder was placed in 10 ml of the selected vehicles in a conical flask. After sealing, the mixtures were shaken at 25 °C for 48 h in a constant temperature shaking incubator (typeTHZ312, Jiangdong Precise Instruments Co., Ltd, Suzhou, China) at 200 r/min and followed by equilibrium for 24 h. Then 5 ml were taken respectively to centrifuge at 5000 rpm for 10 min to separate the undissolved drug and the supernatants were filtered through a Microporous membrane filter (0.45 μm, Tianjin Jinteng Experiment Co., Ltd, Tianjin, China). Filtrates were diluted with methanol and quantified by the HPLC system. The experiment was repeated in triplicates. Results were represented as mean value (mg/ml) ± SD.

2.4. Oil-water partition coefficient study

A certain amount of 6-BBP was dissolved in the n-octanol saturated by water. 4 ml of the solution were placed in a stoppered conical flask, and then 4 ml of the water saturated by n-octanol were added. The mixture was shaken at 25 °C for 24 h until equilibrium in a constant temperature shaking incubator. Then, the upper oil phase was taken to centrifuge at 10,000 rpm for 10 min. The supernatant was quantified by the HPLC system. Three independent experiments were performed to calculate the mean value of the oil-water partition coefficients using the following equation:

$$\log P = \log C_o / C_w \quad (1)$$

where P is the oil-water partition coefficient, C_o is the concentration (mg/ml) of 6-BBP in the n-octanol phase after reaching equilibrium and C_w is the concentration (mg/ml) of 6-BBP in the aqueous phase after reaching equilibrium.

2.5. Pseudo-ternary phase diagram study

A series of blank SNES with varying weight percentages of oil, surfactant and co-surfactant were prepared in this investigation and the pseudo-ternary phase diagram was constructed to identify the self-nanoemulsifying area at 25 °C. The emulsifying properties were assessed using a visual test based on the previous study [21], whereby 0.2 ml of each formulation was added to a glass beaker containing 200 ml of water and gently stirred with a glass bar. The tendency to spontaneously form a nanoemulsion and the progress of droplets spreading were visually evaluated. The nanoemulsion was considered bad when a dull, grayish white emulsion with large oil droplets floating on the surface was formed due to the poor and minimal emulsification of the formulation, especially when stirring was stopped. However, it was qualitatively good when the droplets spread easily and rapidly (within 1 min) in water, forming a fine transparent or slight bluish dispersion [11,22]. Only clear or slight bluish dispersions of particle size 200 nm or lower were considered in the SNES region of the diagram [23]. And then, we carried out the following studies: (1) Influence of various oil concentrations on the globule size with the ratio of surfactant to co-surfactant being 1:1. (2) Effect of the ratio of surfactant to co-surfactant on the globule size and polydispersity index. Finally, the optimized formulation was established. All studies were carried out in triplicates, with very similar observations being made between repeats.

2.6. Preparation of SNES

In the optimized formulation, the level of 6-BBP was 10% w/w of the vehicle. 6-BBP (100 mg/g) was dissolved in the oil phase at 40–75 °C in an isothermal water bath, and the dispersion was gently stirred until the drug was almost dissolved. Then the surfactant and co-surfactant were added and continually stirred till a