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Comparative study on stabilizing ability of food protein, non-ionic surfactant and anionic surfactant on BCS type II drug carvedilol loaded nanosuspension: Physicochemical and pharmacokinetic investigation

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ABSTRACT

Carvedilol (CAR) in its pure state has low aqueous solubility and extremely poor bioavailability which largely limit its clinical application. The aim of the study is to improve the dissolution rate and the bioavailability of CAR via preparing nanosuspensions with different stabilizers. Antisolvent precipitation-ultrasonication technique was used here. Attempts have been made to use food protein- Whey protein isolate (WPI) as a stabilizer in CAR loaded nanosuspension and also to compare its stabilizing potential with conventional nanosuspension stabilizers such as non-ionic linear copolymer-poloxamer 188 (PLX188) and anionic surfactant-sodium dodecyl sulfate (SDS). Optimized nanosuspensions showed narrow size distribution with particle size ranging from 275 to 640 nm. Amorphous state of CAR nanocrystals which also improved the solubility by 16-, 25-, 55-fold accordingly was confirmed by powder X-ray diffraction (PXRD) and differential scanning calorimetry (DSC). From scanning electron microscopy (SEM), flaky shape of PLX188 and SDS nanosuspensions could be revealed but WPI nanosuspension was sphere-shaped. Up to 70% dissolution of loaded drug was observed within 15 min in phosphate buffer (pH 6.8). A pharmacokinetic study in rats indicated that both C_{max} and AUC_{0-36} values of nanosuspensions were estimated to be 2-fold higher than those of reference, suggesting a significant increase in CAR bioavailability.

1. Introduction

It's widely reported that over 40% of potential drugs are poorly soluble in water which has largely hindered their clinical application (Du et al., 2015). Recently, a growing number of new drug delivery systems have been developed to improve the dissolution properties of poorly aqueous soluble chemical entities including employment of co-solvents (Liu et al., 2013; Mishra et al., 2016), cyclodextrins (Vyas et al., 2008), dendrimers (Gupta et al., 2006) and transformation into ionizable drug (Serajuddin, 2007). However, they all still have some drawbacks, for instance, the inclusion technology requires drugs with specific molecular size and weight; whereas the mixed solvent method requires drugs and ingredients with the ability to be dissolved in such organic solvents (Tran et al., 2015) etc.

It is already acknowledged in some literature that the rapidly dissolving drug nanosuspensions can potentially improve the therapeutic efficacy, stability, patient compliance (Ebbesen and Jensen, 2006) and safety (Yang et al., 2008). Nanosuspensions are defined as unique liquid sub-micron colloidal dispersions of nanosized pure drug particles that are stabilized by a suitable polymer and/or surfactant and have a particle size ranging from 1 to 1000 nm (Wang et al., 2013). Nowadays, nanosuspensions have been considered as one of the most promising methods to decrease the particle size and thereby increasing the solubility of poorly water soluble drugs. The main reason behind this claim is that nanosuspensions exhibit the avoidance of organic solvents, advantages of high drug loading, enhanced stability and less toxicity in comparison to other nanocarriers such as liposomes, polymer nanoparticles, lipid nanoparticles and formulations with co-solvents (Ahuja et al., 2014; Shegokar, 2010). Techniques for preparing nanosuspension are classified into top-down and bottom-up types (Mishra et al., 2015). Top-down approaches include high-pressure homogenization (Li et al., 2015) and media milling (Bitterlich et al., 2015; Van Eerdenbrugh et al., 2007) which are based on the high energy process, where drug particles are crushed to reduce the particle size. But their limitation includes energy consumption and environmental contaminations (Chen et al., 2011). The bottom-up approaches include antisolvent precipitation, precipitation-ultrasonication and flash nanoprecipitation (Du et al., 2015). The principle behind these methods is assembling from molecules to nanosized particles (Hu et al., 2014).

Carvedilol (CAR), 1-(9H-Carbazol-4-yloxy)-3-{[2-(2-methoxyphenoxy)

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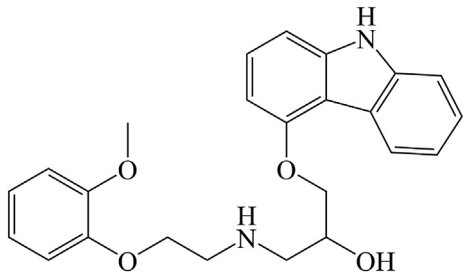

Fig. 1. Chemical structure of carvedilol.

ethyl] amino}-2-propanol, is shown in Fig. 1. CAR is a non-selective beta-blocker (Venishetty et al., 2012) and widely used in clinical practice for the treatment of cardiovascular diseases such as hypertension, congestive heart failure and myocardial infarction (Pamudji et al., 2014). According to the Biopharmaceutical Classification System, CAR is classified as class II drugs with low aqueous solubility and high permeability (Vieth et al., 2004). The solubility in water of CAR is approximately 15 μg/ml, which has limited the clinical applications. At present, many measures have been taken to mitigate this burden.

In this current study, CAR was selected as poorly water soluble drug model and the investigation was focused on preparing CAR nanosuspensions by the method of antisolvent precipitation-ultrasonication (Fig. 2) with three different groups of stabilizers namely food protein-Whey protein isolate (WPI), non-ionic linear copolymer-poloxamer 188 (PLX188) and anionic surfactant- sodium dodecyl sulfate (SDS) to improve the drug dissolution rate and oral bioavailability. Food proteins are amphiphilic in nature and can be used to stabilize nanosuspensions loaded with poorly water soluble drugs (Nejadmansouri et al., 2016). WPI, a byproduct of cheese or casein manufacturing, has been widely used as an ingredient in food products for its high nutritional value and remarkable functional properties (Dai et al., 2016). In this study, we explored the possibility of using the novel food protein WPI to stabilize CAR nanosuspension and compared the stabilizing potential of WPI with that of two different classes of conventional stabilizers such as PLX188 and SDS. The formulations were optimized by comparing particle size, polydispersity index (PDI) and zeta potential. The physicochemical characteristics of the CAR nanosuspensions (WPI-NS, PLX188-NS, SDS-NS) in these optimized formulations were also investigated. Finally, in vitro studies were applied to test the drug dissolution rate. In vivo pharmacokinetic studies in Wistar rats were also carried out to compare the bioavailability of the CAR nanosuspensions with that of the reference preparation.

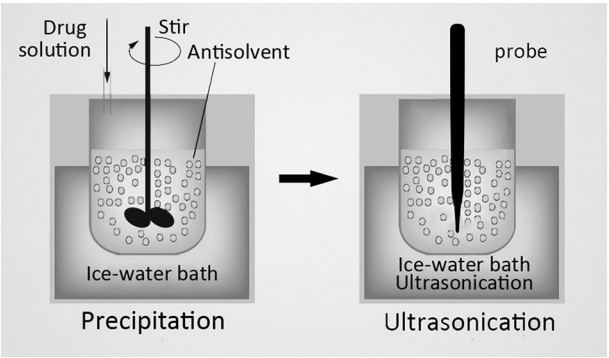

Fig. 2. Schematic illustration of antisolvent precipitation-ultrasonication technique in which drug nanocrystals grow from solution.

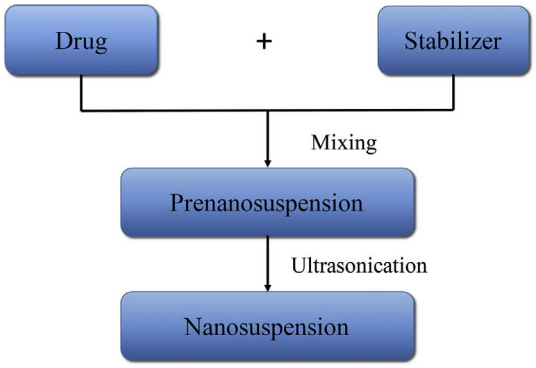

Fig. 3. Flow chart of antisolvent precipitation-ultrasonication technique.

2. Materials and methods

2.1. Materials

Carvedilol (CAR) was provided by Foster Pharmaceutical Company (Hangzhou, China). WPI was purchased from Davisco Foods International Inc. (Le Sueur, MN, USA). PLX188 was procured from BASF chemical company (Ludwigshafen, Germany). SDS was obtained from Suzhou Pinnuo Pharmaceutical Factory (Suzhou, China). Commercial tablets were bought from Qilu Pharmaceutical Co., LTD (Jinan, China). All the other reagents were of analytical grade and used without further purification.

2.2. Methods

2.2.1. Preparation of carvedilol Nanosuspensions

Antisolvent precipitation–ultrasonication technique was applied in this study to prepare CAR nanosuspensions with different types of stabilizers. As shown in Fig. 3. In brief, the organic phase was prepared by dissolving CAR in ethanol (1 ml) and stirred until the drug dissolved completely. Meanwhile, the aqueous phase (10 ml) was prepared by dispersing stabilizers in distilled water. It was previously reported that denatured food proteins have better stabilizing ability than native ones (He et al., 2013). Therefore, WPI was denatured at 90 °C for 40 min before further use. Then, the organic phase and aqueous phase were separately passed through 0.45 μm filter (Xinya purification device factory, Shanghai, China) to remove the possible impurities. After that, cool them in an ice-water bath at 4–8 °C temperature, followed by the rapid addition of the organic phase to the aqueous phase under magnetic stirring for 5 min, thereby causing the drug particles to precipitate immediately from the antisolvent. Then, primary suspensions were treated with an Ultrasonic Processor (JY92-II, Shanghai Xinyi Biotechnology Co. Ltd., China) to achieve smaller particle size after the process of antisolvent precipitation. The period of ultrasound burst was set to 2 s with a pause of 3 s between two ultrasound bursts and the temperature was maintained at 4–8 °C by using an ice-water bath. Then the prepared suspension solution was stirred at the room temperature for 24 h to evaporate the organic solvent.

To evaluate the stabilizing ability of WPI, two different classes of stabilizers such as non-ionic linear copolymer-PLX188 and anionic surfactant-SDS were also investigated. The preparation procedure was similar to that described above except the denaturation step used for food protein WPI.

2.2.2. Optimization of concentrations of drug and stabilizers

To obtain the optimized drug concentration, different formulations were prepared by changing the concentration of CAR and maintaining the concentration of stabilizers, input power and ultrasonication time constant. The same procedure was applied to optimize the concentration of stabilizers. Formulations were prepared as shown in Table 1 and