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Effects of particle size on the pharmacokinetics of puerarin nanocrystals and microcrystals after oral administration to rat

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ABSTRACT

Puerarin, which is extracted from traditional Chinese medicine, is widely used in clinic in China and mainly used as a therapeutic agent to cardiovascular diseases. Owing to its poor water solubility and adverse drug reactions caused by cosolvents after intravenous administration, the development of oral formulation is urgently needed. Nowadays, nanocrystals technique has become a preferred way to develop oral dosage form. In this study, we used high pressure homogenization (HPH) to prepare puerarin nanocrystals and microcrystals with different sizes ranged from 525.8 nm to 1875.6 nm and investigated the influence of particle size on pharmacokinetics. The nanocrystals and microcrystals prepared were characterized using DLS, DSC, XRD and SEM, and we found that the crystalline state of puerarin was changed during the preparation process and the drug was dispersed into HPMC. In the pharmacokinetic study, we observed an increasing of C_{max} and AUC and a decreasing of CL/F with the decreasing of particle size. The AUC of the puerarin nanocrystals (525.8 nm) was 7.6-fold of that of raw puerarin suspension, with an absolute bioavailability of 21.44%. From the above results, we can conclude that nanocrystal technique is an efficient technology to improve the oral bioavailability of puerarin.

1. Introduction

It is reported that at present about 40% of the drugs in the pipelines and up to 70% of molecules coming from synthesis have solubility problems and consequently poor oral bioavailability and delivery problems. What's more, the number of poorly soluble drugs and drug candidates (new chemical entities) is steadily increasing (Keck et al., 2008). In order to overcome this natural shortcoming, researchers have developed a large amount of techniques, e.g. solubilization, solvent mixtures, inclusion compounds, complexation and so on. But these formulation techniques can only be used to a certain number of drugs exhibiting special features, moreover, low drug loading was arisen in such methods (Keck and Müller, 2006).

Nanocrystal technology was first introduced into pharmaceutical field in the early time of 1990s, and quickly got researchers' attention from then on. To date, there are several commercial drug products based on drug nanocrystal technology and more than twenty products are in different clinical stages (Gao et al., 2012; Keck et al., 2008). Basically, nanocrystals is a material crystallization in nano-size, stabilizers were added in the prepared process, although which is not needed in principle, to achieve a higher stability or a longer shelf life (Wang et al., 2012a). Bottom-up (precipitation) and top-down (disintegration) approach are the two techniques to produce drug nanocrystals, because of the drawbacks like wide size distribution and low stability, the bottom up techniques are not really widely used. In the top down technologies, pearl/ball milling was widely used before and there were several products, but a general problem of the method was potential erosion of material from the milling pearls leading to product contamination, so high pressure homogenization (HPH) is more frequently used nowadays (Gao et al., 2008; Keck and Müller, 2006).

Puerarin (Fig. 1), a major active ingredient of *Pueraria lobata* extraction, has gained much attention of researchers. Pharmacological studies have showed that this compound has numerous biological activities, such as antioxidant, hepatoprotective, estrogenic effects (Zhang et al., 2006; Xia et al., 2013) and anticancer activity (Wang et al., 2013). At present, puerarin is mainly used as a therapeutic agent to cardiovascular diseases (including myocardial ischemia, angina pectoris, and hypertension and so on) (Chen

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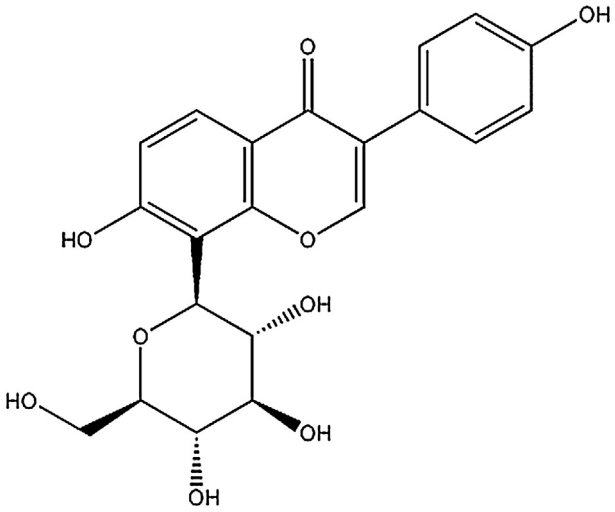

Fig. 1. The chemical structure of puerarin.

et al., 2012). As a Class IV drug in Biopharmaceutics Classification System (BCS), the only clinical administration route of puerarin is intravenous administration. Due to the low solubility of puerarin in water, 50% (v/v) 1,2-propanediol was added into the current puerarin injection formulation as a cosolvent. The 1,2-propanediol and its metabolites may be one of the sensitizing agents, which may lead to side effects, such as pruritus, chest tightness and shortness of breath (Chen et al., 2010). In addition, owing to the short elimination half-life of puerarin in human beings, frequently intravenous administration of high doses may be needed, possibly leading to severe and acute side effects (Luo et al., 2011). To reduce or eliminate these side effects, other administration route, especially the oral administration which is regarded as the most common and safest mode of administration is urgently needed.

To puerarin, many technologies such as phospholipid complex (Li et al., 2006), solid lipid nanoparticle (Luo et al., 2011) and self-emulsifying system (Quan et al., 2007; Zhang et al., 2012), have been used to improve its solubility in water, thus enhance oral bioavailability, but unfortunately, none of these techniques can improve the bioavailability to a large extent. Nowadays, nanocrystal technology is proven to be a technique which could improve the bioavailability of same drugs by many times (Gao et al., 2012). The only work that using nanocrystal technique on puerarin was published in 2012 (Wang et al., 2012b), but this work only focused on intravenous administration. In this paper, we investigated the influence of particle size on the oral pharmacokinetics of puerarin and the potential to develop an oral dosage form of puerarin nanocrystals.

2. Materials and methods

2.1. Materials

Puerarin (purity > 99%) was purchased from Nanjing Zelang Pharmaceutical Co., Ltd., China. Hydroxypropyl methyl cellulose (HPMC) was supplied by Sigma, America. P-hydroxybenzoic acid (analytical grade) was purchased from China National Medicines Corporation Ltd. All other materials and reagents were of analytical grade and ultra-pure water was used throughout this study. The specific pathogen free grade male Sprague-Dawley (SD) rats were purchased from the Experimental Animal Center of Shanghai University of Traditional Chinese Medicine (Shanghai, China). The animals were kept at a temperature of 23 ± 2 °C under natural light/dark conditions for 1 week and were given with food and water ad libitum. The animal experiment was evaluated and approved by the Animal Ethics Committee of Shanghai University of T C M.

Table 1
Preparation parameters of microcrystals and nanocrystals.

Formulations	Sheer speed (rpm) and time (min)	Homogenization pressure (bar) and cycle
Microcrystals	19,000, 10	0
Nanocrystals	19,000, 10	1500 × 20

2.2. Preparation of different puerarin formulations

Puerarin nanocrystals were prepared using the high pressure homogenization method (HPH), with the same formula components but varied process parameters as showed in Table 1. Puerarin (400 mg) was added into 40 ml 1% (w/v) HPMC-water solution at 70 °C under magnetic stirring. The obtained mixture was then disintegrated into smaller particles by high shear homogenizer at 19,000 rpm for 10 min using Fluko® FA25 (FLUKO, Germany) at the same temperature and microcrystals was gained. Then the microcrystals were homogenized at 1500 bar for 20 cycles at room temperature to obtain the nanocrytals. The nanocrystals were lyophilized for further use.

2.3. Characterization of the formulations

2.3.1. Particle size analysis

Dynamic light scattering (DLS) method was used to determine the particle sizes, without any dilution at 25 °C. The measurements were carried out in PSS. Particle sizing systems (PSS, America) and all samples were analyzed in triplicate.

2.3.2. Morphology study

Scanning electron microscopy (SEM) was employed to evaluate the morphology of puerarin nanocrystals. The morphology analysis was carried out under a S-4800 Field emission scanning electron microscope (Hitachi, Japan), after the sample was coated with gold on a holder and dried in vacuum. The electron beam characteristics were 20 mA for 80 s and 10 kV or 15 kV in the experiments.

In the investigations of nanocrystals, freeze dried powders without any cryoprotectant added were used to avoid the observation of a larger particle size due to the encapsulation of nanocrystals by cryoprotectant, as reported by a paper published before (Wang et al., 2012a).

2.3.3. Crystalline state study

Generally, X-ray diffraction (XRD) and differential scanning calorimetry (DSC) were the two major means to determine the crystal form and crystallinity. In this paper, the two methods were performed as below:

In the XRD study, a vertical goniometer (Model RINT2000) was used and the crystallinity of the sample was examined by using a Ni filtered Cu Kα radiation source (D/MAX-2550 V, Rigaku Co., Japan). During the measurement, continuous scanning model (2-theta/theta) was selected with a scanning rate of 10°/min and 2-theta ranged from 10° to 80° using a step size of 0.02°. The generator was set to 40 kV and 100 mA and the radiation wavelength is 1.542 Å. After measurement, data was analyzed by MDI Jade 5.0 software, and the smooth curves were gained after the elimination of the influence of noise by setting the Savitzky–Golay Filter to 11 points.

The thermal properties, including the transition temperatures such as the melting and crystallization temperatures of different powders were determined by DSC (TA Analysis, 2910 MDSC V4.4E) in a dry nitrogen atmosphere. For the DSC measurement, the