Development and in-vivo assessment of the bioavailability of oridonin solid dispersions by the gas anti-solvent technique

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\textbf{A B S T R A C T}

We developed solid dispersions, using the gas anti-solvent technique (GAS), to improve the oral bioavailability of the poorly water-soluble active component oridonin. The solubility of oridonin in supercritical carbon dioxide was measured under various pressures and temperatures. To prepare oridonin solid dispersions using the GAS technique, ethanol was used as the solvent, CO\textsubscript{2} as the anti-solvent and the hydrophilic polymer polyvinylpyrrolidone K17 (PVP K17) was used as the drug carrier matrix. Characterization of the obtained preparations was undertaken using scanning electron microscopy (SEM), X-ray diffraction (XRD) analyses and a drug release study. Oridonin solid dispersions were formed and oridonin was present in an amorphous form in these dispersions. Oridonin solid dispersions significantly increased the drug dissolution rate compared with that of oridonin powder, primarily through drug amor-
phization. Compared with the physical mixture of oridonin and PVP K17, oridonin solid dispersions gave higher values of AUC and \textit{C}_{\text{max}}, and the absorption of oridonin from solid dispersions resulted in 26.4-fold improvement in bioavailability. The present study illustrated the feasibility of applying the GAS technique to prepare oridonin solid dispersions, and of using them for the delivery of oridonin via the oral route.

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\section{Introduction}

Oridonin (Fig. 1) is an active component extracted from \textit{Rabdosia rubescens}. Oridonin has been shown to have marked anti-cancer effects. It has been used in the treatment of primary liver cancer, carcinoma of the esophagus, and pancreatic cancer (Zhang and Ren, 2003). The commercially available tablets or syrups are crude extracts with a very low content of oridonin (<1.5%) (Ji et al., 1999). Oridonin is poorly soluble in water so, in general, the oral bioavailability of this agent is very low. In recent years, various oridonin preparations have been investigated. These include oridonin self-microemulsifying drug-delivery systems (SMEDDS) (Zhang et al., 2008), solid lipid nanoparticles (Zhang et al., 2005), and nanosuspensions (Gao et al., 2007).

The use of solid dispersions is a common and popular approach to enhance the dissolution rate of poorly water-soluble drugs. A drug is highly dispersed in the carriers of solid dispersions. The reported mechanisms of solid dispersions for improving the dissolution and bioavailability involve various drug–carrier inter-

actions such as solid solutions and amorphous precipitates; carrier
hydrophilicity; and aggregation reduction (Sammour et al., 2006).

There are various methods to prepare solid dispersions, including melting, solvent evaporation and solvent melting techniques, and supercritical fluid (SCF) technology. In recent years, SCF technology has attracted attention due to its four major advantages over conventional methods (Shariati and Peters, 2003). First, the particles may be obtained without using organic solvents or under high temperature. Second, the particles may possess a smaller size, a lower size distribution and good fluidity. Third, the size and morphology of the particles may be controlled by operation parameters. Fourth, SCF technology simplifies the number of particle-producing stages to mainly one step; it therefore avoids the phase transition, high surface energy and chemical degradation that occur during conventional procedures. Of the various SCF technologies (i.e., rapid expansion of supercritical solution (RESS), gas anti-solvent (GAS), solution-enhanced dispersion by supercritical fluids (SEDS)), the GAS technique is a very promising method to prepare solid dispersions (Patomchavivat et al., 2008). It utilizes SCFs to expand the organic solution containing the substance to be micronized, which decreases the solvency power of the organic solution and results in the formation of fine microparticles (Shariati and Peters, 2003).

In the present study, to enhance the dissolution rate and bioavailability of oridonin, oridonin solid dispersions were prepared by the GAS technique with supercritical CO\textsubscript{2} as the
Fig. 1. Chemical structure of oridonin.

anti-solvent. Furthermore, characterization of the prepared oridonin solid dispersions was carried out by scanning electron microscopy (SEM) and X-ray diffraction (XRD). In-vitro dissolution tests and in-vivo assessment of bioavailability were carried out to investigate the promotion of the action of dissolution and bioavailability enhancement of oridonin.

2. Materials and methods

2.1. Materials

Oridonin was purchased from Hangzhou Huadong Medicine Group Kangrun Pharmaceutical Company Limited (Hangzhou, China). The oridonin content was 97% according to the manufacturer. The polymer polyvinylpyrrolidone K17 (PVP K17) was provided by BASF (Hanover, Germany). Other chemicals were of analytical grade. The autoclave (Φ 40 mm × 160 mm) was designed by Shanghai Chemical Industry Design Institute Company Limited (Shanghai, China). The supercritical pilot plant was produced by NOVA (Geneva, Switzerland).

2.2. Solubility determination

Oridonin solubility in supercritical CO₂ was measured in a semi-continuous flow-type apparatus (Fig. 2A) according to a previous report with some modification (Rafael and Arturo, 2001). Oridonin powder was introduced into the buffer cell and the equilibrium cell. This was followed by connecting the system and checking the tightness of the system. CO₂ was pre-cooled to 0°C by a liquid-cooled bath. The pre-cooled CO₂ was then delivered to the equilibrium cell by a gas booster pump. The pressure gauge displayed pressure in the equilibrium cell and control the CO₂ flow rate. The temperature controlling system was switched on until it reached the pre-determined temperature. CO₂ was pumped to the system continuously. When the temperature and pressure reached the required values, system pre-saturation was undertaken. Then, CO₂ was fed to the buffer cell and equilibrium cell for 1 h at a constant flow rate. Subsequently, the equilibrated CO₂ passed by two needle valves and flowed into the sample-taking device. The latter comprised a phase separator and a second vial which was used to release the pressure gradually and to maintain a steady pressure. Needle valves 2 and 3 controlled the pressure during sample-taking. The mass flow volume of CO₂ could be obtained by the difference in the volume before and after sampling as shown at the wet-flow meter.

After the experiment, the lines and valves placed after needle valve 2 were removed and rinsed thrice with ethanol to remove any remaining extract. After ethanol was recovered, the resultant residue was dissolved with methanol. The obtained sample was used for oridonin analyses by high-performance liquid chromatography (HPLC). HPLC analyses were undertaken in an Agilent system (HP 1200, Agilent, Santa Clara, CA, USA). The experimental site was an ODS column (Kromasil C18, 250 mm × 4.6 mm, 5 μm). The mobile phase was methanol/water (60:40, v/v) with a flow rate of 1.0 mL/min. The column temperature and detector were 25 °C and 242 nm, respectively. To determine the influence of CO₂ flow speed, presaturate time, temperature and pres-
sure on oridonin solubility, the experiments were conducted under the following conditions: CO₂ flow speed of 0.5, 1, 1.5, 2, 3 mL/min; presaturation time of 0.5, 1, 1.5, 2 h; temperature of 308.15, 313.15, 318.15, 323.15, 328.15 K; pressure of 8, 10, 12, 14, 16 Mpa.

2.3. Preparation of solid dispersions of oridonin

Oridonin solid dispersions were prepared by the GAS technique using the setup shown in Fig. 2B. Oridonin powder and the hydrophilic polymer PVP K17 were mixed thoroughly. The mixture was dissolved in ethanol. The obtained solution was introduced into the vessel, followed by closing the vessel. The temperature controlling system was activated until the temperature increased to 55 °C. Then CO₂ was fed into the vessel at 55 °C until the pressure reached 14 MPa. The temperature and pressure were maintained at 55 °C and 14 MPa for 15 min to pre-saturate the system. Subsequently, CO₂ was pumped into the vessel continuously by the pressure controlling pump, and flowed out gradually through a needle valve to keep the pressure stable and expedite sample-taking. After CO₂ had flowed for 45 min, the pressure was released rapidly and the product in the vessel collected and stored for further use.

2.4. SEM observation

SEM micrographs were taken using a JSM-6360LV Environmental Scanning Electron Microscope (JEOL, Tokyo, Japan). Samples were coated with gold before examination (cathode dispersion). The accelerating voltage was 20 kV. The samples were observed at 1000 × magnification.

2.5. XRD analyses

XRD analyses were carried out using an X-ray diffractometer (D/MAX 2550 VB/PC, Rigaku, Japan). The experimental condition was: CuKα radiation; 2θ was from 3° to 50° with steps of 0.02°; voltage was 40 kV; and current was 100 mA.

2.6. In-vitro dissolution test

The release of oridonin from oridonin powder and solid dispersions was determined using Chinese Pharmacopoeia Release Method III. The dissolution test was undertaken using 200 mL of distilled water as a dissolution medium to achieve sink condition at 100 rpm and 37 °C. A 1.0-mL aliquot of sample was withdrawn at a pre-determined time of 5, 10, 15, 30, 60, 90 min and replaced with the same volume of fresh medium. The sample was filtrated through a membrane filter (0.45 μm). The content of oridonin in the filtrate was determined by HPLC.

2.7. In-vivo assessment of the bioavailability of oridonin

The study protocol was approved by the Institutional Animal Ethical Committee of the Shanghai University of Traditional Chinese Medicine (Shanghai, China).

Four male beagle dogs (8–10 kg) were supplied by the Laboratory Animal Center of the Shanghai University of Traditional Chinese Medicine. The animals were starved for 12 h before the experiment but had free access to water. A crossover study was carried out with a washing out period of 1 week.

The physical mixtures of oridonin with PVP K17 and oridonin solid dispersions were filled into the capsule. Each dog was orally...
administered one test capsule. Two milliliters of blood sample were collected before dosing and 15, 30, 45, 60, 120, 180, 240 min after dosing. Plasma was obtained by centrifugation at 4000 rpm for 10 min. All samples were kept at −20°C until use. Fifty micro-liters of phosphate-buffered solution (PBS, pH 5.0) was added to 0.5 mL of dog plasma, and the mixture vortex-mixed for 10 s. The resulting sample was mixed with 3 mL of ethyl acetate, followed by vortex-mixing for 3 min. It was then centrifuged for 10 min at 4000 rpm. The supernatant layer (2 mL) was evaporated under an atmosphere of N₂ at 55°C. After vortex-mixing for 30 s, the sample was centrifuged at 12,000 rpm for 10 min. Twenty microliters of supernatant were withdrawn for HPLC determination. The regression equation of the calibration curve of oridonin was 
\[ A = 15.257C + 4.1444 \] (correlation coefficient \( r = 0.9989 \)) at 0.1–4.0 \( \mu \)g/mL, where \( A \) represents the peak area and \( C \) represents the plasma concentration of oridonin. The limit of detection (signal-to-noise ratio (S/N) > 3) was 40 ng/mL. At concentrations of 0.8, 2.4, and 4 \( \mu \)g/mL, the recovery was 51.47 ± 4.84%, 57.65 ± 8.18% and 62.05 ± 3.11%, respectively. The inter and intra-day precision of the assay was <8.53% and <9.70%, respectively. The pharmacokinetic parameters were analyzed by non-compartment analyses.

2.8. Statistical analyses

Values were mean ± SD. Statistical analyses were carried out by one-way ANOVA. \( p < 0.05 \) was considered significant.

3. Results and discussion

3.1. Solubility detection of oridonin in supercritical \( \text{CO}_2 \)

In general, the GAS technique is applied if a drug has limited solubility in SCFs such as supercritical \( \text{CO}_2 \) (Garay et al., 2010). To ascertain the viability of the GAS technique in preparation of oridonin solid dispersions, we initially conducted the solubility detection of oridonin in supercritical \( \text{CO}_2 \), and investigated the influence of various experimental conditions such as pre-saturation time, \( \text{CO}_2 \) flow velocity, temperature and pressure upon oridonin solubility.

Fig. 3A shows the influence of pre-saturation time on the solubility of oridonin in supercritical \( \text{CO}_2 \). The solubility increased slightly at 0.5 h compared with that at 1 h, and there was little change in solubility for the pre-saturation time from 1 h to 2 h. Taking the efficiency into consideration, we selected a pre-saturation time of 1 h. With respect to the influence of the flow velocity of \( \text{CO}_2 \) on solubility, although a slight increase in solubility from 0.5 mL/min to 1 mL/min was observed, a decrease in solubility was found from 1 mL/min to 3 mL/min (Fig. 3B). We therefore selected a flow velocity of 1 mL/min. We also evaluated the influence of temperature and pressure on oridonin solubility in supercritical \( \text{CO}_2 \). The solubility of oridonin in supercritical \( \text{CO}_2 \) decreased with increasing temperature (Fig. 3C), whereas it increased with increasing pressure (Fig. 3D). The dissolving capacity of a substance in supercritical \( \text{CO}_2 \) is dependent upon the density of supercritical \( \text{CO}_2 \), and the density of supercritical \( \text{CO}_2 \) increases with decreasing temperature and increasing pressure (Shi et al., 2009). Therefore, our results were in accordance with these phenomena. In all cases, the solubility of oridonin in supercritical \( \text{CO}_2 \) was <3 mg/mol, indicating the feasibility of applying the GAS technique in the preparation of oridonin solid dispersions.

3.2. SEM observations

Fig. 4 shows the SEM images of PVP K17 and oridonin solid dispersions. The morphology of oridonin solid dispersions was clearly different to those of PVP K17. Oridonin solid dispersions were much larger and more adhesive compared with PVP K17.

3.3. XRD analyses

We investigated the solid state of oridonin to observe changes in crystal morphology using XRD analyses. From the XRD patterns shown in Fig. 5, the sharp peak at a 2θ value of 8° appeared for the mixture of oridonin and PVP K17, whereas it disappeared in the case of oridonin solid dispersions. Other decreases or the disappearance of signals in oridonin solid dispersions compared with the mixture...
of oridonin and PVP K17 were also noted. The XRD pattern of oridonin solid dispersions suggested a decrease in crystallinity and its existence in the amorphous state, which indicated the formation of a solid dispersion system. In addition, it has been suggested that the amorphous form plays an important part in solubility and the dissolution rate which, in general, results in higher solubility and a faster dissolution rate (Kim et al., 2008).

3.4. In-vitro dissolution test

The dissolution profiles of oridonin powder as well as solid dispersions prepared by the GAS method are shown in Fig. 6. Oridonin powder exhibited ~20% dissolution in the first 5 min, whereas at the same time point the solid dispersions showed 66% dissolution. The dissolution rate remained constant after 30 min of dissolution for both samples. These results demonstrated that the solid dispersions improved in dissolution rate compared with the raw drug. The presence of oridonin in amorphous form in the solid dispersions might contribute to the higher dissolution rate. As previously reported, rapid hydration of hydrophilic polymers promotes the drug wetting process in aqueous surroundings and enlarges the surface area between the solid and water, which may also be an additional factor responsible for the improved dissolution rate in solid dispersions (Park et al., 2010). Furthermore, it was reported that a reduction in particle size had a role in the improvement of dissolution rate (Moneghini et al., 2001). Based on the dissolution results, one could suggest that the improved dissolution rate may result in better oral absorption.

3.5. In-vivo bioavailability study

The in-vivo study was undertaken to investigate the pharmacokinetic behavior of oridonin after oral administration of a mixture of oridonin and PVP K17 and solid dispersions. Fig. 7 shows the plasma profiles of oridonin in male beagle dogs after oral administration of the two preparations at 10 mg/kg. The plasma concentration of oridonin after oral administration of the mixture of oridonin and PVP K17 could not be detected in the first 45 min or after 120 min due to its limited absorption. The plasma profiles of oridonin solid dispersions demonstrated a considerable improvement in oridonin absorption compared with the mixture of oridonin and PVP K17 (Table 1). The maximum plasma concentration ($C_{\text{max}}$) and the area under the concentration–time curve ($AUC_{0-\infty}$) values of oridonin after oral administration of solid dispersions were significantly higher than those of the mixture of oridonin and PVP K17 ($p < 0.05$). The relative bioavailability was calculated using the following equation:

$$F = \frac{AUC_{\text{test}}}{AUC_{\text{reference}}} \times 100\%$$

The absorption of oridonin from solid dispersions resulted in a 26.4-fold increase in bioavailability compared with the mixture of oridonin and PVP K17. Therefore, oridonin solid dispersions prepared by the GAS method could provide a promising strategy for improving the bioavailability of oridonin. The enhanced bioavailability of oridonin in solid dispersions could be attributed to the improved rate of dissolution and absorption rate.

4. Conclusions

The present study demonstrated a method for the development of oridonin solid dispersions using the GAS technique for oral administration. The prepared solid dispersions resulted in a considerably improved rate of drug dissolution compared with that of oridonin powder, which was related to the amorphization of oridonin as revealed by XRD analyses. With the enhanced dissolution of oridonin, the oral bioavailability of oridonin solid dispersions was dramatically increased to 26.4-fold that of the mixture of oridonin and PVP K17. These results promote the potential use of solid dispersions for the delivery of oridonin via the oral route.

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