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Abstract

Physical Characterization and In-vivo Evaluation of Oleanolic Acid Powders Processed by Spray Freeze Drying (SFD) Technology

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Oleanolic acid (OA), a pentacyclic triterpenoid compound, is commonly present in Chinese herbs, such as *Fructus Ligustri Lucidi, Fructus Forsythiae* and *Radix Ginseng*, etc. It has been shown to exhibit anti-inflammatory, antitumor, hypoglycemic and protecting liver activities. Oleanolic acid has been used as an OTC drug for oral administration to treat human liver disorders in China. The commonly used formulations are tablets, capsules and pills.

Oleanolic acid belongs to a BCS Ⅳ active compound according to the biopharmaceutics classification system in FDA guidance, which shows poor aqueous solubility and low permeability across the intestinal membrane. It was reported that the solubility of OA was only 4.61×10^{-3} μg/ml at room temperature with slow dissolution rate. Furthermore, the permeability study across Caco-2 cell monolayer showed the P_{app} of OA was $1.1\text{-}1.3\times10^{-6}$ cm/s, which was similar to that of a low permeable marker atenolol ($Papp=0.25\times10^{-6}$ cm/s). The poor solubility and permeability lead to the low oral bioavailability (i.e., 0.7% at oral doses of 25 and 50

mg/kg in rats). The aim of the present study is to increase the oral bioavailability of OA by improving its dissolution rate as well as the membrane permeability by preparation of solid dispersions using Spray Freeze Drying (SFD) technology and salt formation methods.

To enhance the dissolution of OA, spray freeze drying technology and salt formation methods were utilized to prepare the solid dispersion of OA. SFD is a relatively new technology used to prepare solid dispersion powder under low temperature conditions, which is especially suitable for heat-sensitive materials with poor aqueous solubility. Moreover, the salt formation is an effective method for improving the dissolution of poor water soluble drugs. To improve the permeability across the intestinal membrane of OA, absorption enhancer was used and evaluated by transport study on a Caco-2 cell monolayer model and in-vivo pharmacokinetic study.

According to the dissolution results, PVP40 was selected as the excipient. To prepare SFD OA-PVP40 solid dispersion powder, the spray freeze drying apparatus was firstly installed. Power X-ray diffraction (PXRD) showed that all SFD powders lacked the characteristic crystalline peaks, indicating a completely amorphous OA had been prepared. Scanning electron microscopy (SEM) showed that SFD OA-PVP40 particles were porous particles composed of many small subunits with a geometric diameter of about 1-5 μm. The dissolution rate of OA raw material was slow. Only ~ 70% OA could be dissolved within 2 hours. On the contrary, ~ 90% OA could be dissolved within 10 mins for all of the SFD processed OA-PVP formulations. Accelerated stability test demonstrated both SFD OA-PVP40 at 1:1 and 1:9 ratios (w/w) were stable for 6 months under 40°C with 75% relative humidity. And no significant differences were detected by comparison the dissolution profiles at 1, 2, 3

and 6 months to the initial samples.

Meanwhile, a LC-MS and a liquid-liquid extraction method for OA were established. The bioavailabilities of commercial OA tablet and SFD OA-PVP 1:1 were evaluated in SD rats. SFD powders displayed higher C_{max} and AUC (153% and 268.56%, respectively) compared to the commercial OA tablets. However, there were no significantly differences between these two formulaes, which may suggest that the very low permeability of OA is the rate-limiting step for OA absorption.

To prepare more bioavailable OA formulation, sodium caprate was used as a potential absorption enhancer. And the sodium salt of OA was prepared to enhance solubility of OA. The new formulations were SFD OA:PVP:SC 1:1:2 and SFD OANa:PVP:SC 1:1:2. PXRD and SEM revealed that both powders were completely amorphous and had a porous morphology with small columnar connections. The dissolution rate is higher than those of other formulations. More than 58% and 75% OA was dissolved in 10 min, 60% and 84% in 2 h, respectively. It has been demonstrated that sodium caprate can greatly increase the transported amount of OA from 3.68% to 10.16% in 2 hous. Compared to OA tablet, both two formulations had shorter T_{max} (12.5 and 16 min, respectively) and higher C_{max} (399.36 and 348.91 ng/ml, respectively), which are significantly different from those of the OA tablet. Furthermore, the relative bioavailability of SFD OANa:PVP:SC was 218%, compared to the OA tablet. In conclusion, the combined use of SFD technology, salt formation and absorption enhancer can successfully improve the dissolution rate, intestinal membrane permeability and oral bioavailability of OA.

Keywords: Oleanolic acid; Solid dispersion; Spray freeze drying; Absorption enhancer; Caco-2 cell model; Bioavailability; Dissolution; Stability study