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Enhancement of dissolution and permeability of 3',4'-dimethoxy flavonol-3-O-β-D glucopyranoside by preparing its solid dispersions using citric acid as matrix

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ABSTRACT:
3',4'-Dimethoxy-flavonol-3-O-β-D-glucopyranoside (GDH) is a candidate drug, which can reduce cholesterol, protect myocardium and has anti-tumor effect. However, the solubility and permeability of GDH are low, which results in poor oral bioavailability. In this work, the SDs of GDH (GDH-CA_1 and GDH-CA_2) were prepared in order to enhance its solubility and permeability. Some small molecular acids were screened as the matrix of SDs by the solubility method and then citric acid was used to prepare the GDH SDs.The prepared GDH SDs were characterized by a series of methods, Such as differential scanning calorimetry (DSC), thermogravimetric analysis (TGA), X-ray powder diffraction (XRPD) and Fourier transform infrared spectroscopy (FTIR).Subsequently, the solubility, dissolution and permeability of the SDs of GDH were studied. GDH was amorphous in the prepared SDs. GDH-CA_1 and GDH-CA_2 revealed a significantly higher solubility and dissolution rate compared with GDH. In situ single-pass intestinal perfusion experiments indicated that GDH-CA_1 could enhance the permeability of GDH.

KEYWORDS: Poorly Water-Soluble Drug, Solid Dispersion, Solubility, Permeability.

I. INTRODUCTION

Solubility is a key factor that determines the degree of drug absorption[1]. The bioavailability of drugs with poor solubility is low, which affects therapeutic effect. According to research, about 40% of marked drugs and 90% of drugs to be developed have low solubility[2]. Therefore, it is urgent to solve the problem of low solubility. The solubility of drugs is mainly related to the structures, crystal forms and particle size of drugs. At present, there are many methods to improve the solubility of drugs, such as co-crystal, polymorphism, salt and solid dispersion[3-6]. Solid dispersion (SD) is a technical method that can significantly improve the solubility of poorly water-soluble drugs. It is a dispersion system in which drugs are uniformly dispersed in solid matrix in different forms[7-8]. In SD the drug is highly dispersed in carrier, which can prevent the drug from aggregation, decrease lattice energy and increase wettability. At the same time, some carriers can inhibit the recrystallization of amorphous drugs, thus effectively improving the dissolution rate of drugs[9-10].

3',4'-Dimethoxy-flavonol-3-O-β-D-glucopyranoside (GDH) is a novel synthesized compound and patented in China. GDH has many pharmacological effects, such as reducing blood lipid, protecting myocardium and anti-tumor[11]. The monohydrate of GDH (Fig. 1) is its stable form. We expect that the solubility and intestinal permeability of GDH can be improved by preparing its solid dispersions. We had used different polymers (HPMCAS-LF, HPMCAS-MF and HPMCAS-HF) as hydrophilic matrixes to prepare the amorphous solid dispersions of GDH by the solvent evaporation method[12]. The result showed that the amorphous solid dispersion of GDH could not significantly improve the solubility of the drug, and amorphous drug was prone to phase transition and crystallize during storage[13].

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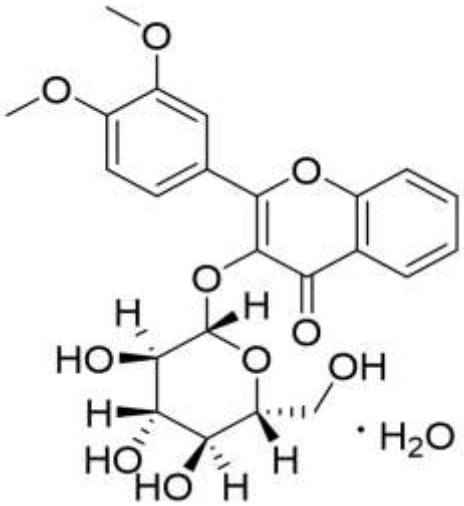

Fig. 1 Chemical structure of the monohydrate of GDH

Singh had developed a new method using small molecular excipients as hydrophilic matrixes to improve the solubility and dissolution rate of low water-soluble drugs. Aqueous solutions containing large amounts of malic acid, tartaric acid and citric acid can solubilize haloperidol through acid-base interaction. When these concentrated aqueous solutions were dried, SD of haloperidol could be obtained and haloperidol was dispersed in these weak acids in an amorphous form. The SD dissolved rapidly in the whole pH range of gastrointestinal tract, which can effectively improve haloperidol dissolution[14]. Recently we have found that the concentrated ethanol solution of citric acid can solubilize GDH effectively. So we will use citric acid as a hydrophilic matrix to prepare the SD of GDH, which can improve GDH solubility and absorption.

1. Materials and methods

1.1. Materials

The monohydrate of GDH (≥ 98%) was supplied by Eight Plus One Pharmaceutical Co., Ltd. (Shanghai, China). Citric acid(CA, 99.5%) was purchased from Aladdin biochemical T**e**chnology Co., Ltd. (Shanghai, China). Methanol (HPLC grade) was obtained from Tiandi Co., Ltd. (Anhui, China). Ethanol, ethyl acetate, isopropanol were analytically pure and purchased from Xilong Science Co., Ltd. (Guangdong, China).

1.2. The screening of matrix

Tartaric acid, glutaric acid, niacin, niacinamide, fumaric acid, malic acid and citric acid were screened by the solubility method. The same amount of above compounds was dissolved in ethanol respectively. The solubility of GDH in the resulted solution was investigated. The results showed that GDH had the highest solubility in the solution of CA. So CA was used as the matrix to prepare the SDs of GDH.

1.3. Preparation of GDH SDs

GDH-CA_1: CA (5g) was dissolved in 30 mL anhydrous ethanol, and GDH (0.5g) was added to the above solution. The mixture was heated until completely dissolved. The resulted solution was evaporated at surrounding temperature. The remained solid was dried under vacuum at 40℃ for 24 hours to obtain GDH -CA_1. 1 g GDH and 5 g CA were used to prepare GDH-CA_2 by the same method.

1.4. Differential scanning calorimetry (DSC)

The solid dispersions of GDH, CA and GDH were analyzed by DSC (STA449-F3, Netzsch, Germany). An empty aluminum pan was used as a reference. 2-5 mg of samples are weighed and put into an aluminum pan with a lid.Nitrogen flow rate was 20 ml/min. All samples were scanned from 25 ℃ to 300 ℃ at a speed of 20℃/min, and the obtained curves were recorded and analyzed.

1.5. X-ray powder diffractometry (XRPD)

The crystallinity of GDH, CA and the prepared solid dispersions was analyzed by Bruker D8 ADVANCED Discover X-ray diffractometer (Bruker AXS Inc., Madison, WI, USA). The instrument uses CuKα radiation, the tube voltage and current is 40 kV and 40 mA respectively. The sample is scanned at a speed of 10 °/min in the range from 3°to 45°2θ. A step size is 0.02s. The obtained data were analyzed.

1.6. FT-IR spectroscopy

The FT-IR spectrophotometer (Nicolet Impact 410, Thermo Fisher, MA, USA) is used to investigate the interaction between GDH and CA. All samples were ground into uniform powder with KBr at the ratio of 1:100, and pressed into tablets. The scanning range of the sample is 4000-400cm^{-1}, and the number of scans is 128.

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1.7. Equilibrium solubility

The excess samples were added to 10 mL of 50 mM pH 6.8 PBS. The mixtures were stirred at 37 °C and 150 r/min for 24 h. Then 3mL sample was withdrawn and centrifuged at 13000 rpm for 10min. The supernatant was diluted and analyzed by HPLC.

1.8. In vitro dissolution

A certain amount of GDH or the prepared solid dispersions were placed in 200 mL of 50 mM pH 6.8 PBS at 37 °C, and the stirring speed was 150 rpm. 3 mL solution was taken out at certain intervals (1, 5, 10, 20, 30, 45, 60, 90, 120, 180, 240, 1440 min) and the fresh dissolution midea with the same temperature and volume was added. Samples were centrifuged for 10 minutes and the supernatant was withdrawn and diluted. GDH concentration was determined by HPLC.

1.9. In situ single-pass intestinal perfusion (SPIP) studies

The animal experiments were operated strictly according to the regulations of the Animal Ethics Committee of Guilin Medical College. 250-300g male SD rats were chosen. Before the experiment, the rats were fasted for 12 hours and only water was supplied. Then the rats were anesthetized by intraperitoneal injection of 20% urethane solution of 5mL/kg and fixed on a heated operating table to keep a body temperature of 37 ℃. Along the abdomen midline an incision of 3–4 cm was made. A 10 cm proximal jejunal segment was taken out and cannulated on two ends with PVC tubing. The wet dressing was used to keep the exposed intestinal segment moist. The 300 μg/mL GDH-CA_1 perfusion solution was prepared by dissolving a certain amount of GDH-CA_1 in 200 mL 50 mM pH 6.8 PBS. GDH was added to 50 mM pH 6.8 PBS to prepare 100 ug/mL GDH perfusion solution. All the prepared perfusion solutions were pumped through the intestinal segment at 37 °C respectively. Firstly the intestinal segment was washed with blank perfusion PBS at a flow rate of 0.5 mL/min. The prepared perfusion solutions were then pumped through the intestinal segment at a flow rate of 2.5 mL/min for 1 h to obtain steady state. Subsequently the pre-weighed glass tubes was used to collect 6 samples at an interval of 10 min. GDH concentration in the perfusion samples was investigated by HPLC. After the experiment was done, an intracardiac injection of saturated potassium chloride solution was performed according to the protocol for euthanasia of experimental animals. The length and radius of intestinal segments were accurately measured.

Water absorption was determined by the gravimetric method. The outlet concentration of GDH was corrected by the following equation:

$$C'_{out} = C_{out} \times \frac{Q_{out}}{Q_{in}} \quad (1)$$

Where C'_{out} is the corrected outlet concentration of GDH; C_{out} is the concentration of GDH; Q_{in} and Q_{out} are inlet and outlet perfusion flux (mL/min) respectively.

The effective permeability (P_{eff}) through the intestinal segment was determined by the following equation:

$$P_{eff}\ (\mathrm{cm/s}) = \frac{-Q_{in} \ln (C'_{out}/C_{in})}{2\pi RL} \quad (2)$$

Where C_{in} is the inlet concentration of GDH; R is the radius of the intestinal segment, and L is the length of the intestinal segment.

1.10. HPLC

The concentration of GDH drug was determined by Agilent 1260 HPLC instrument(Agilent Technologies, Santa Clara, CA). The specific conditions were as follows: mobile phase methanol: water = 1:1 (volume ratio), chromatographic column: A Zobrax SB-C_{18} column (150 mm × 4.6 mm, 5 mm), column temperature: 35 ℃, iso-elution, flow rate: 1 mL/min, detection wavelength: 341nm, injection volume: 5 μL.

1.11. Statistical analysis

All surveyed data are showed as mean ± standard deviation (SD). SPSS software was used for analysis of variance (ANOVA) and Tukey tests, $P < 0.05$ is termed significant.

II. RESULTS AND DISCUSSION

2.1. DSC-TG

As shown in Fig. 2, GDH shows a melting peak at 200 °C. GDH has a broad endothermic peak at 149 °C, which indicates that GDH loses a water molecule. CA showed a melting peak at 160 °C, which is consistent with the

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reported melting point of CA in the literature[15]. GDH-CA_1 has a melting peak at 160℃, which is the same as the melting peak of CA, and no endothermic peak is observed near the melting point of 200 ℃. These results indicate that GDH in SD is amorphous. GDH-CA_2 shows a peak at 159℃, which can be assigned to CA melt. No melting peak of GDH indicates that GDH is also amorphous in the GDH-CA_2 SD.

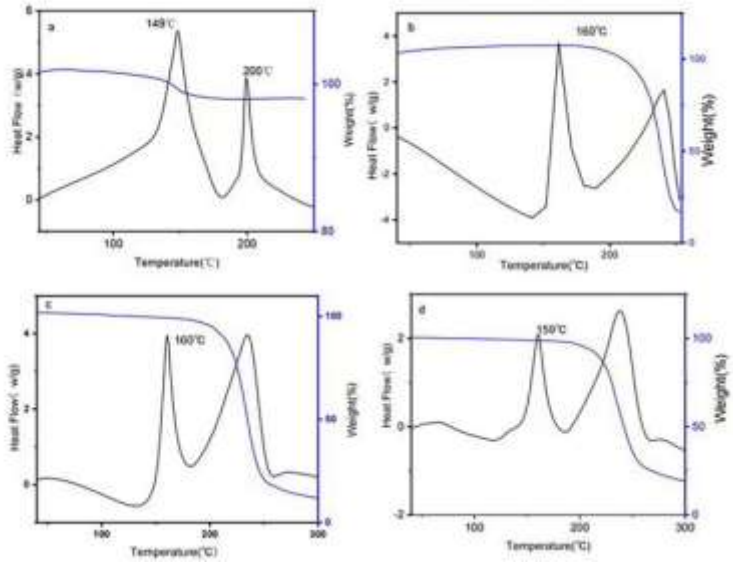

Fig. 2 DSC-TG curves of GDH (a), CA (b), GDH-CA_1 (c) and GDH-CA_2 (d)

2.2. XRPD

Powder X-ray diffraction is an effective method for characterizing solid dispersions[16]. As shown in Fig. 3, GDH had strong characteristic diffraction peaks at 8.52°,10.5°,9.6°,15.58° and 19.96° 2θ, while the diffraction peaks of CA are at 14.29°,16.2°,18.07°,24.13° and 26.23° 2θ. The characteristic diffraction peaks of GDH disappeared in the GDH-CA_1 and GDH-CA_2 SDs, and the diffraction peaks of CA remain in these SDs. The XRPD data indicate that the original crystal structure of GDH is disrupted in the GDH-CA_1 and GDH-CA_2SDs, and GDH is amorphously dispersed into the carrier of CA[17]. These results are consistent with the DSC curves.

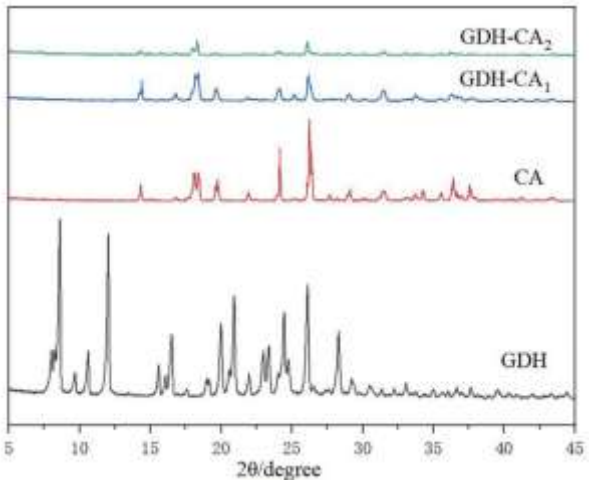

Fig. 3 XRPD diffraction patterns of GDH, CA, GDH-CA_1 and GDH-CA_2

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2.3. FT-IR

FT-IR can be used to identify specific functional groups and its location[18]. As shown in Fig. 4, GDH and CA show strong absorptions at 1607 cm^{-1} and 1689 cm^{-1}(C=O stretch) respectively. The C=O stretch of CA in GDH-CA_1 and GDH-CA_2 shifts to 1712 cm^{-1} and 1724 cm^{-1}respectively, and these peaks become more sharp. These results suggest that there are hydrogen bonds between the carboxyl group of CA and the carbonyl group of GDH. GDH has a strong absorption at 3456 cm^{-1}(O-H stretch). This peak for GDH-CA_1 and GDH-CA_2 shifts to 3467 cm^{-1} and 3476 cm^{-1}, respectively. These shifts indicate that intermolecular interactions exist between GDH and CA.

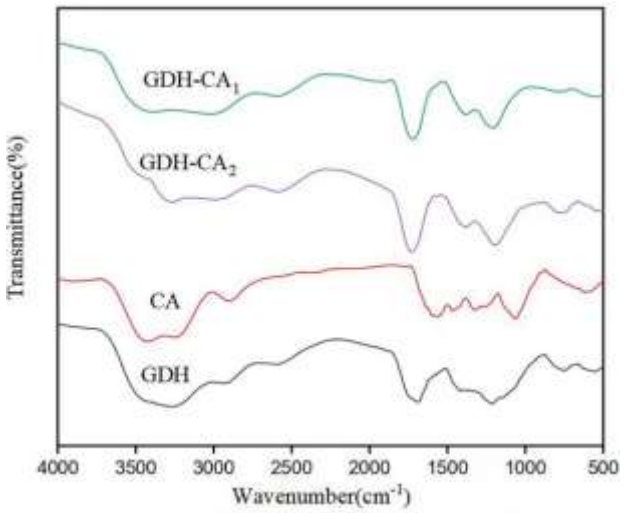

Fig. 4 FT-IR spectra of GDH, CA, and the prepared SDs

2.4. Equilibrium solubility

As shown in Fig. 5, the equilibrium solubility of GDH is about 139 μg/mL. The solubility of GDH-CA_1 and GDH-CA_2 was 596 μg/mL and 289 μg/mL, which are about 4 and 2 times higher than that of GDH respectively. Because the free energy of the amorphous drug in SDs is larger than the crystalline one, and the solubility of the prepared SDs is higher[19]. GDH-CA_2 has lower solubility than GDH-CA_1, which can be ascribed to its high drug loading and low wettability.

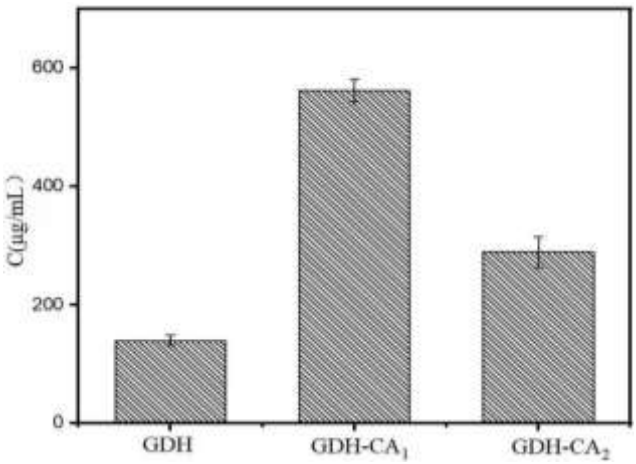

Fig. 5 Equilibrium solubility of GDH and the prepared SDs

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2.5. In vitro dissolution

As shown in Fig. 6, the dissolution of the prepared SDs exhibits "spring" behavior. The concentration of GDH-CA_1 and GDH-CA_2 solid dispersions reach 750 μg/mL and 560 μg/mL within 1 min respectively, which are about 7 and 6 times higher than that of GDH respectively. This result shows that the dissolution of GDH can be enhanced by preparing GDH-CA SDs. GDH is highly dispersed in the carrier in amorphous form, which has high surface free energy. So there is no need to overcome the lattice energy during dissolution[20]. The rapid dissolution of CA can change the microenvironment around SD particles, which may enhance the dissolution of GDH.The GDH concentration of GDH-CA1 and GDH-CA2 decreased significantly after 5min, which was due to the oversaturation of the solution,leads to the crystallization of GDH[21]. This result also demonstrates that CA cannot inhibit the crystallization of GDH effectively. The dissolution rate of GDH-CA_1 is faster than that of GDH-CA_2, indicating that the high drug loading is unfavorable to GDH dissolution. This conclusion is agreement with the report of other papers about SD[22]. For GDH-CA_2, more GDH molecules aggregate as CA is dissolved and are prone to crystallize when they are in contact with dissolution media.

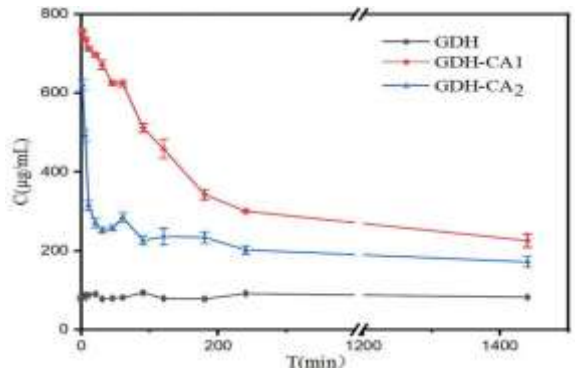

Fig. 6 Dissolution curves of GDH drug solution, GDH-CA_1 and GDH-CA_2

2.6. Effect of the prepared SDs on permeability of GDH

The method of in vivo intestinal perfusion in rats is easy to operate, and there is a good correlation between its absorption and human intestinal absorption[23]. GDH and GDH-CA_1 could be dissolved in 50 mM pH 6.8 phosphate buffer solution to obtain the perfusion solutions of 300 μg/mL. The intestinal permeability of these solutions was investigated by SPIP. As shown in Fig. 7, the permeability coefficient valueof GDH 9.8, and that of GDH-CA_1 is 17.9. So GDH-CA_1 has high permeability than GDH and it can enhance GDH absorption. This result can be attributed to the fact that the supersaturated solution formed by dissolving GDH-CA_1 can promote transmembrane transport of GDH for its high chemical potential.

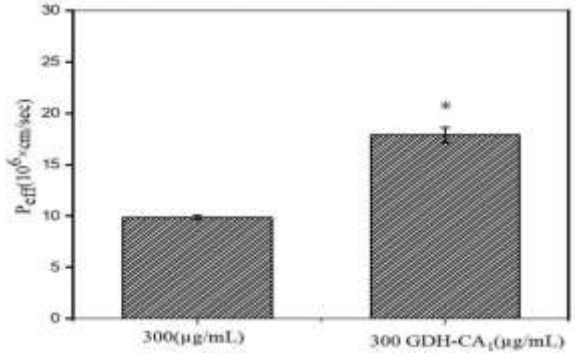

Fig. 7 SPIP studies of GDH and its SD. *p <0.01 versus GDH solution. Average±SD; n=4

III. CONCLUSION

Two SDs of GDH based on CA matrix had been prepared by Solvent evaporation method and characterized by DSC-TG, XRPD and FT-IR. The solubility, dissolution and intestinal permeability had been investigated. The results show that GDH is dispersed amorphously in CA matrix. There are strong intermolecular interactions between GDH and CA. The prepared SDs can significantly improve the solubility and dissolution of GDH. As the GDH loading increase, the solubility and dissolution decrease. GDH-CA_1 can enhance intestinal permeability of GDH, which may be due to the formation of supersaturation.

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