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Preparation and pharmacokinetic study of fenofibrate cubic liquid crystalline

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ABSTRACT

An LCC delivery system for Fenofibrate (Fen) was developed to improve its poorly oral bioavailability. Fen-LCC preparation methods were screened, and the prepared Fen-LCC was then characterized by a polarizing microscope and transmission electron microscopy (TEM). The spray drying technique was selected to dry and solidify particles into powder. The *in vitro* release of Fen-LCC was measured and *in vivo* pharmacokinetic experiments were carried out on rats after oral administration. Particles prepared through the high-temperature input method exhibited structural characteristics of LCC, and re-dissolved particles maintained the same features. The LCC delivery system can significantly improve *in vitro* release outcomes. After oral administration, AUCs of the suspension and LCC systems were measured at 131.6853 μg·h/ml and 1435.72893 μg·h/ml, respectively. The spray drying process presented here better maintains cubic structures, and the LCC system significantly improves bioavailability levels.

1. Introduction

Fenofibrate (Fen) is a phenoxyaromatic acid that can regulate blood lipid levels [1]. After administration, Fen is hydrolyzed by esterases of tissues and plasma, generating the bioactive metabolite fenofibric acid (Fefa) and thus playing a role in reducing blood lipid levels.

Fen is a lipophilic compound that is almost insoluble in water with an oil/water distribution coefficient of 5.3, resulting in its poor oral absorption. Therefore, the bioavailability of oral Fen administration is very low [2,3]. The solubility must

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be improved in order to increase its bioavailability *in vivo*. Solubilized nanoparticles have clinical defects such as poor physical and chemical stability, so that it would be difficult to practice in the normal situations [4]. The development direction of nanoparticle is to solidify the nanoparticles, then to re-dissolve and re-structure them. Therefore, spray drying and freeze drying technologies are extensively applied in the solidification of nanoparticles, among which freeze drying technology would cost too much and does not suit the industrial production. Fen preparations used in foreign and domestic markets include capsules and tablets, and the micronization of bulk drugs is the most commonly used approach. In addition, other dosage modes (e.g., double-layer osmotic pump preparation [5], delayed-release micropellets [6], nanosuspension and micronized capsules of Fen) have been reported in the literature. However, due to solubility limitations, certain factors restrict the improvement of its bioavailability.

In this study, a high-temperature input method was applied to prepare Fen lipid cubic liquid crystalline (LCC) particles. Worle's results [7,8] indicate that non-cubic will transfer to cubic during autoclaving of the dispersions. Given the advantages of this unique "honeycomb" structure (the closed-lipid-bilayer structure composed of dual-consecutive water and lipid layers) and the large membrane surface area it provides, LCC can increase the solubility of Fen, thereby improve its bioavailability. The obtained Fen-LCC microparticles were spray-dried and solidified into the powder for the ease of administration, transport and carrying.

2. Materials and methods

2.1. Materials

Fen raw material (Wuhan Galaxy Chemical Co., Ltd., Hubei, China); Fen standard (Batch No: 200401, National Institute for Food & Drug Control, Beijing, China); glycerol monooleate (GMO, Aladdin, Shanghai, China); poloxamer 407 (F127, Badische Anilin- und Soda-Fabriken Co., Ltd., German).

SYQ DSX 280B Autoclave (Shanghai Shenan Medical Instrument, Shanghai, China); XSP-8CA Polarizing Microscope (Shanghai Optical Instrument Factory No. 6, Shanghai, China); JEM-2010HR Transmission Electron Microscope (JEOL Co., Ltd., Japan); L-117 Mini-Lab Spray Dryer (Beijing Laiheng Science&Trade Co., Ltd., Beijing, China); Zetasizer Nano ZS90 Laser Particle Size Analyzer (MALVEM, England); LC-20AB HPLC (Shimadzu International Trading Co., Ltd., Shanghai, China); UH-S2 Ultrasound Cells Crusher (AUTO SCIENCE Technology Co., Ltd., Taiwan, China); ZRD6-B Drug Dissolution Instrument (Shanghai Huanghai Pharmaceutical Instrument Factory, Shanghai, China).

SD rats, weighed 250 ~ 300 g, without gender limitations, were provided by Animal Center, Guangzhou University of Chinese Medicine, Certificate of Conformity: SCXK (Yue) 2008-0020. All animal procedures were approved by the Animal Ethics Committee of Guangdong Pharmaceutical University.

2.2. LCC preparation

First, 20 g GMO and 2 g F127 were melted in a 60 °C water bath, and then 1 g Fen was added, melted and mixed into an 80 °C water bath; 30 min of sonication was then performed to form a uniform blend; 50 ml of distilled water was then added to the mixture and processed under high pressure levels and 121 °C for 15 min; the mixture was then subjected to Ultrasound Cells Crusher treatment for 30 min to shrink it to a nanometer-size stable state [9]. After the above procedures, a stable dispersion of Fen-LCC was successfully prepared.

2.3. LCC precursor preparation

2.3.1. Spray dry precursor preparation

The prepared Fen-LCC was added to 12.42% maltodextrin and then diluted with water (1:10) for the following spray drying procedure. The spray drying conditions were as follows: the inlet air temperature was set to 130 °C, the actual outlet temperature was set to 97~90 °C, the wind speed was set to 90 cm/sec, and the feeding rate was set to 35 ml/min. Spray drying generated white spray-dried Fen-LCC powder.

2.3.2. Determination of in vitro release

Three copies of 100 mg Fen raw materials (100 mesh) and spray-dried Fen-LCC powder (including 100 mg Fen) were tested for *in vitro* release. We used 500 ml of 1% SDS solution as the releasing medium, and the test was performed at 37 °C and 100 rpm. We sampled 4 ml of solution at the 0, 1st, 3rd, 5th, 7th, 9th and 11th h, and 4 ml of fresh releasing medium was added at the same time. The solution was filtered through a 0.45 μm pore membrane to discard the initial filtrate, and the subsequent filtrate was collected, from which 20 μl was injected into HPLC for determination [10,11]. The detection wavelength was measured at 286 nm, and the mobile phase was methanol-water (82:18). The column temperature was measured at 30 °C with a flow rate of 1.0 ml/min. Peak areas were used to calculate concentrations at different time points.

2.4. Polarizing microscope observations of Fen-LCC

Nine different GMO: H_2O-ratio groups were selected, namely: 90:10, 80:20, 70:30, 60:40, 50:50, 40:60, 30:70, 20:80 and 10:90. Fen and GMO ratios were selected uniformly from 6:94 to 1:99 [12]. GMO was mixed with F127 at a ratio of 10:1 and then melted in a 60 °C water bath. The melted mixture was added to a corresponding proportion of Fen and then melted and mixed into an 80 °C water bath and sonicated for 30 min to achieve uniformity. A high pressure treatment was then applied at 121 °C for 15 min [13], generating a stable dispersion of Fen-LCC. The above stable LCC dispersion with different dosages of Fen was applied in droplets onto a slide for observation under a polarizing microscope.

2.5. TEM characteristic observation

A droplet of Fen-LCC sample solution was distributed onto a glass slide covered in a copper mesh with formvar support film. After being left to stand for 1.5 min, excessive liquid has been absorbed and the copper mesh had dried; 2 droplets of 2% tungstophosphoric acid hydrate ($H_3(PO_4W_{12}O_{36}) \cdot 14H_2O$, pH = 7) were then added onto the slide and covered with the sample slide for 1.5 min of negative staining. The copper mesh was

then removed and placed on numbered filter paper. Once the copper mesh had dried, it could be observed through TEM [14,15] (Fig. 3). The LCC had a particle size of roughly 100 ~ 300 nm.

2.6. *Pharmacokinetic studies*

2.6.1. *Medication program*

12 SD rats were weighed, and divided into 2 groups according to the principle of weight proportionality, with 6 in each group. All the animals were fasted 12 h before the experiment, while with free access to water. One group was set as the control group, administrated Fen suspension by oral route, while the other group was set as the sample group with the administration of re-dissolved solution of spray-dried Fen-LCC powder by oral route. Fen suspension preparation: 50 mg maltodextrin was dissolved in 3 ml distilled water in a 60 °C water bath, and then 40 mg Fen was added, mixed into stable mixture. The administration dosage was 20 mg/kg body weight.

2.6.2. *Blood sample collection*

The rat orbital venous blood was obtained at the 0.5, 1.5, 2.5, 4.5, 7, 10, 13, 16, 24, 30, 36, 48 and 60 h after the administration, respectively, and placed into 0.8 ml heparinized tube for the centrifugation at 4000 r/min for 10 min. The supernatant was stored in –80 °C refrigerator for future use.

2.6.3. *Analysis of plasma concentration*

300 μl plasma sample was quantitatively obtained and placed into 2 ml plastic centrifuge tube, added and mixed by 1000 μl acetonitrile for the deproteinization. The tube was then vortexed for 30 sec and centrifuged at 4000 r/min for 10 min. The supernatant was then transferred into a transparent glass tube, and dried with nitrogen purge at 37 °C. The residue was then re-dissolved with 300 μl methanol, vortexed 2~3 min and filtered with 0.45 μm organic membrane. 20 μl filtrate was injected into HPLC [16,17]. The chromatographic conditions were as the following [18]: column: Kromasil C_{18} (4.6 mm × 250 mm, 5 μm), protected by DIKMA Easy Guard II C_{18} (4.6 mm × 10 mm); mobile phase: methanol-water-10% phosphoric acid (75:24:1, pH = 4.0); detection wavelength: 286 nm; column temperature: 30 °C; flow rate: 1.0 ml/min.

Table 1 – Pharmacokinetics parameters of cubosomes and suspension for fenofibrate.

Parameters	Cubosomes	Suspension
$t_{1/2}$ (h)	9.3189	11.7903
t_{max} (h)	4.6750	5.1700
C_{max} (μg/ml)	88.6018	6.5973
$AUC_{(0\rightarrow60)}$ (μg·h/ml)	1435.7289	131.6853

2.6.4. *Data processing*

According to the plasma drug concentration-time curve, 3P97 was used for the pharmacokinetic calculation procedures, one chamber fitting was performed towards the concentration-time of both raw material and LCC, and the data were shown in Table 1.

3. Results and discussion

3.1. *Characterization of Fen-LCC by polarizing microscope*

LCC is optically isotropic material; it will be a dark field when observed under a polarizing microscope. In Fig. 1, 1a shows a physical mixture of Fen in which highlighted colors denote the presence of Fen; 1b shows a condition involving an LCC drug overdose, spurring crystallized Fen precipitation (this is denoted by the highlighted color); 1c shows that LCC generates a dark field without Fen overdose; 1d presents a photograph of the lamellar phase (namely at 9:1 and 8:2 GMO: H_2O ratios) that shows a bright silver color distinct from the bright color generated as a result of Fen overdose. These two different colors and shapes made it easy to distinguish them through a polarizing microscope.

Fig. 2a shows that LCC nanoparticles were analogically spherical and uniform and did not adhere to one another; Fig. 2b presents an enlarged image illustrating that the particles showed features of cubic crystalline extended toward the 3D space while the lipid bilayer exhibited a twisted folded 3D tight structure with circular permutations, a minimal surface, and a unique internal dual waterway structure (the black parts in Fig. 2b).

3.2. *Particle size inspection after re-dissolvation*

The Fen-LCC and spray-dried Fen-LCC were re-dissolved for particle size determination. The results show that particle sizes

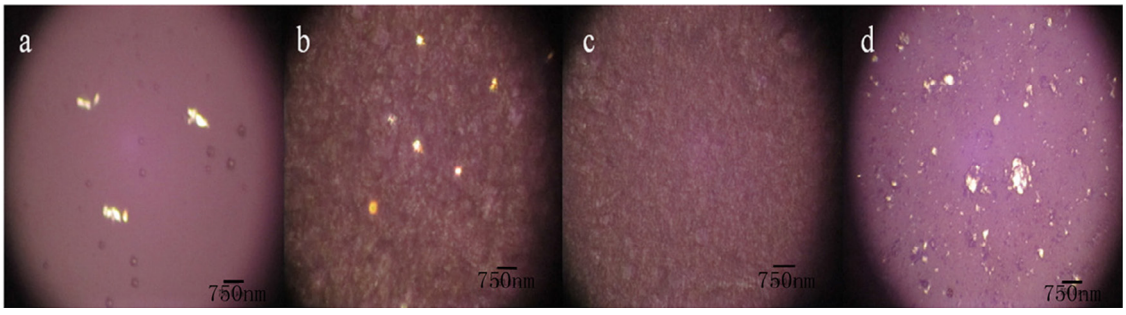

Fig. 1 – Photograph of Fen-LCC by polarizing microscope. a: photograph of simple mixture; b: photograph of cubosomes with excess fenofibrate; c: photograph of cubosomes; d: photograph of lamellar phase.

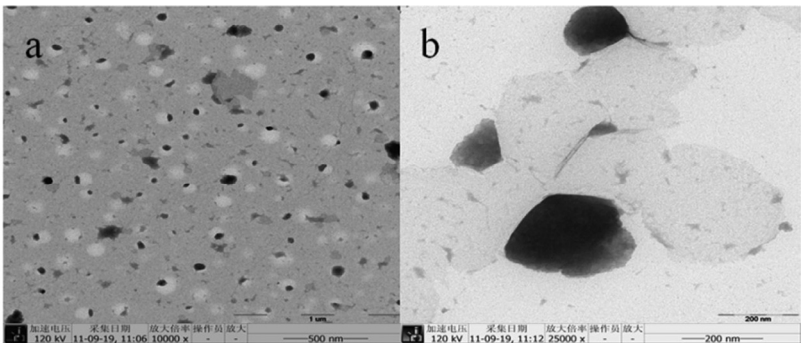

Fig. 2 – TEM photograph of cubosomes. a: TEM photograph of cubosomes; b: enlarged TEM photograph of cubosomes (the dark parts in Fig. 2b represent water layer and the grey parts represent lipid layer).

increased from 235.2 ± 25.32 nm to 276.5 ± 38.18 nm following re-dissolvation. It showed that the particle size did not change much after solidification.

3.3. *Features of spray-dried products after re-dissolvation*

The re-dissolved solution of spray-dried Fen-LCC showed a dark field under the polarizing microscope and black square blocks under TEM with a particle size of roughly 200 nm surrounded by white objects that may have been accessories (Fig. 3). It showed that spray-dried products also had the "honeycomb" structure.

3.4. *In vitro releasing results*

From the results (Fig. 4), it can be inferred that the rate of LCC release was significantly improved relative to that of the raw material. The accumulative release of Fen-LCC reached 80.46% by the 5th h but only 38.82% for the raw material; at the 11th h, the release of the 2 preparations tended to become balanced at accumulative release levels of 99.53% and 44.36%,

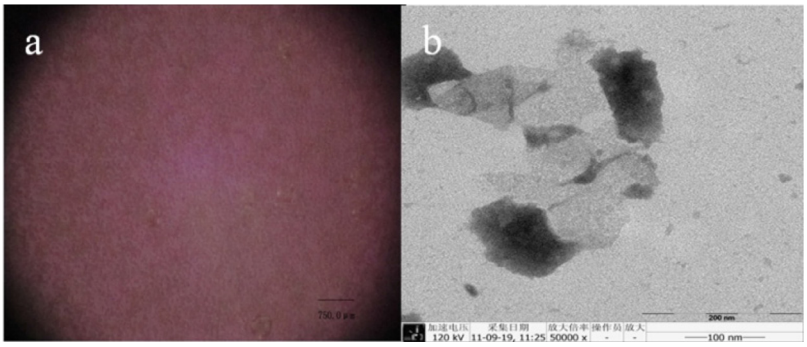

Fig. 3 – Photograph of spray-dried cubosomes after resolvation. a: polarizing microscopic photograph; b: TEM photograph.

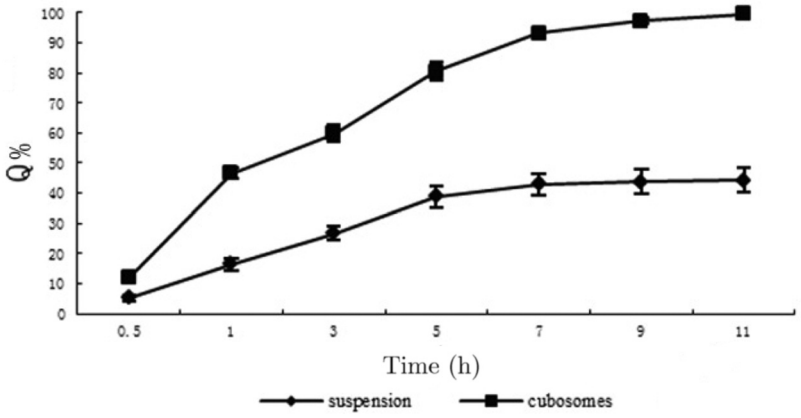

Fig. 4 – Accumulated releasing of cubosomes and suspension (n = 3).

respectively, indicating that Fen-LCC can significantly increase release rates and quantities.

3.5. *Pharmacokinetic study*

3.5.1. *Specificity*

The results showed that the internal standard and plasma endogenous substances would not interfere with the drug content determination.

3.5.2. *Standard curve*

The concentration (μg/ml) was performed weighted least squares regression calculation towards Fefa peak area/internal standard peak area (A_F/A_N), the regression equation was as the following: $A_F/A_N = 0.09339C + 0.01421$ ($R^2 = 0.9966$); a good linear relationship of Fefa could be obtained in the range of 0.4~200 μg/ml, with the detection limit as 150 ng/ml.

3.5.3. *Accuracy*

Extraction recovery. The extraction recoveries were 97.96%, 107.04% and 108.46% towards the low, medium and high concentrations, respectively, indicating that the sample pretreatment method was reasonable and the extraction rate was within a specified range.

Method recovery. The average recoveries of low, medium and high concentrations were 97.58%, 93.72% and 94.91%, respectively. The method recoveries were greater than 90%, with RSD% < 10%, and could be used for the biological sample determination.

3.5.4. *Precision*

Results of the intra- and inter-day precision showed that the intra-day RSD% were 7.45%, 3.46% and 1.75% and the inter-day RSD% were 4.92%, 6.09% and 3.56% for low, medium and high-concentration samples, respectively. The RSD% were all less than 10%, indicating that the method had good precision and could be used for the determination of biological samples.

3.5.5. *Data processing*

The results were showed in Table 1 and Fig. 5. T_{max} of Fen-LCC and raw material were 4.6750 and 5.1700 h, respectively; $t_{1/2}$ were 9.3189 and 11.7903 h, revealing that LCC could promote the releasing rate when compared with the raw material. C_{max} were 88.6018 μg/ml and 6.5973 μg/ml, respectively, indicating that when compared with the raw material, LCC could release the drug and generate the efficacy much quicker; $AUC_{(0\rightarrow60)}$ were 1435.7289 μg·h/ml and 131.6853 μg·h/ml, respectively, certifying that LCC could significantly increase the absorption situation of Fen, thereby greatly improving its bioavailability. All the above pharmacokinetic parameters revealed that the concentrations of rat blood drug were significantly increased, indicating that Fen-LCC could significantly increase the adsorption in rats, thus improving the bioavailability.

4. Conclusions

In this study, the successful application of spray drying technology broadened the development of nanoparticles solubilization areas. LCC could increase the solubility and *in vitro* releasing of poorly soluble drugs, thus improving their bioavailability, which might provide certain experimental significance for the use of poorly soluble drugs [19].

The amphiphilic molecule has a special molecular structure, which could gather and form a variety of ordered structures in solvents. In the aqueous solution, with the changes of amphiphilic molecule concentrations, the system would appear micelles, vesicles, liquid cubic crystal, hexagonal crystal, lamellar liquid crystal, *etc*. As a stabilizer, F127, when the ratio increases from 3% to 15%, could turn the lipid cubic crystalline from the spiral structure to the cubic structure. When the F127 ratio reaches 10%, it would make the lipid cubic crystalline much more stable, therefore, the ratio of oil phase/F127 would normally be prescribed as 10:1.

LCC is optically isotropic material, presenting entirely dark field within the polarizing microscope. Fen has poor solubility in water, forming crystals in water and showing colorful highlights under the polarized light, so it could be identified with a polarizing microscope. When Fen is overdosed, it would crystallize in the aqueous phase, exhibiting colorful highlights. If Fen is not overdosed, it would be completely encapsulated in LCC, showing completely dark vision under

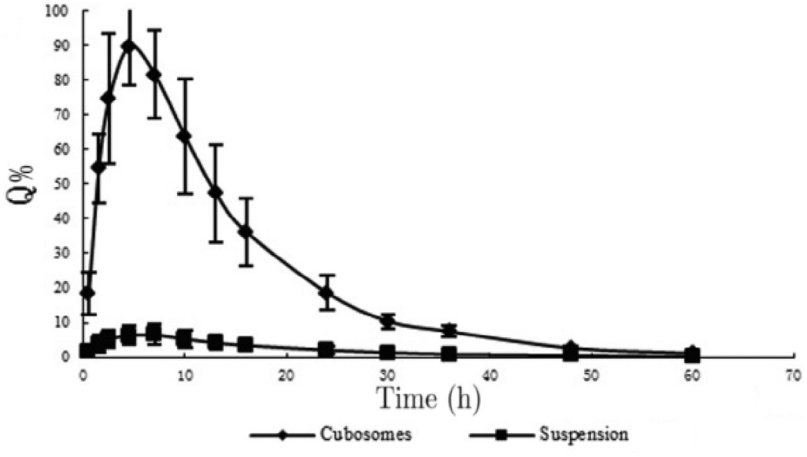

Fig. 5 – The plasma concentration-time profiles of the cubosomes nanoparticles and the suspension in rats (n = 6).

the polarizing microscope. So the polarizing microscope could be used to identify whether Fen is overdosed or not with the polarizing microscope.

Conflicts of interest

The authors declare that there are no conflicts of interest.

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