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ORIGINAL ARTICLE

Preparation of fenofibrate immediate-release tablets involving wet grinding for improved bioavailability

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Abstract

Objective: The purpose of this study was to investigate the dissolution and oral bioavailability of an immediate-release tablet involving wet grinding of a poorly water-soluble drug, fenofibrate. *Methods:* The milled suspension was prepared using a Basket Dispersing Mill in the presence of a hydrophilic polymer solution and then granulated with common excipients, and compressed into an immediate-release tablet with blank microcrystalline cellulose granules. *Results:* Compared with unmilled tablets (56% within 30 minutes), the dissolution of wet-milled tablets (about 98% in 30 minutes) was markedly enhanced. No significant decrease in the dissolution rate (96% in 30 minutes) of the wet-milled tablet was observed after 3 months under 40°C and 75% relative humidity storage. In addition, the oral bioavailability of the wet-milled tablets (test) and Lipanthyl® supra-bioavailability tablets (reference) was determined in beagle dogs after a single dose (160 mg fenofibrate) in a randomized crossover, own-control study. The results suggested that both the area under the plasma concentration–time curve ($AUC_{(0-t)}$ = 46.83 ± 11.09 μg/mL h) and the mean peak concentration of the test (C_{max} = 4.63 ± 1.71 μg/mL) were higher than the reference ($AUC_{(0-t)}$ = 35.12 ± 10.97 μg/mL h, C_{max} = 2.11 ± 0.08 μg/mL). The relative bioavailability of the wet-milled tablet was approximately 1.3-fold higher. Furthermore, the apparent rate of absorption of fenofibrate from the wet-milled tablet (T_{max} = 2.63 hours) was faster than that from Lipanthyl® (T_{max} = 3.75 hours). *Conclusion:* These results indicated that the dissolution and the bioavailability of fenofibrate were significantly enhanced by wet-grinding process. So, this shows that wet grinding is a powerful technique to improve the bioavailability for poorly water-soluble drugs, especially for Biopharmaceutics Classification System Class II compounds.

Key words: *Dissolution; fenofibrate; hydrophilic polymer; oral bioavailability; particle size reduction; wet grinding*

Introduction

Improvement of the oral bioavailability of poorly water-soluble drugs remains one of the most challenging aspects of drug development[1]. However, the bioavailability after oral administration can often be improved by enhancing the dissolution rate of such insoluble drugs, especially for Biopharmaceutics Classification System (BCS) Class II compounds[2,3]. Although many approaches, such as solid dispersion, salt formation, solubilization, and particle size reduction, have commonly been used to increase the dissolution rate, all these techniques have potential limitations. The solid dispersion technique involves recrystallization during storage, poor reproducibility, and limited opportunity for scale-up of manufacturing processes. Salt formation can only be used for weakly acidic or basic drugs and not for neutral ones. The use of cosolvents or surfactants to improve the dissolution rate poses problems, such as patient compliance and commercialization[1,4]. According to the Noyes–Whitney equation, reducing the particle size would increase the overall surface area, which would subsequently increase the dissolution rate of a poorly water-soluble drug[5]. Nevertheless, particle size reduction usually produces an amorphous powder with poor wettability and stability (e.g., aggregation) during storage. Accordingly, it is essential to solve the problem of stability. So, in this study we have chosen hydroxypropyl methyl cellulose (HPMC) as a grinding carrier to reduce the mobility of fenofibrate particles and prevent

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aggregation during the storage process, mainly involving the binding of the drug to the carrier[6].

Fenofibrate {2-[4-(4-chlorobenzoyl)phenoxy]-2-methyl-propanoic acid, 1-methylethyl ester}, is an effective lipid-lowering agent as it decreases low-density lipoprotein and very-low-density lipoprotein levels, and increases high-density lipoprotein levels. However, its oral bioavailability is very low (about 30% in humans) because it is practically insoluble in aqueous media. Fenofibrate can be categorized as a BCS Class II drug based on its hydrophobic/lipophilic ratio (MW = 360.831 g/mol, log P = 5.575;)[7]. Thus, the dissolution rate of fenofibrate is expected to limit its absorption from the gastrointestinal tract. Several methods, such as the preparation of solid dispersions, cyclodextrin inclusion complexes, adsorption onto silica by supercritical carbon, and particle size reduction, have already been attempted to improve its dissolution[6,8]. Among these, particle size reduction has been shown to substantially enhance the dissolution of fenofibrate, and higher bioavailability has been reported for micronized fenofibrate compared with nonmicronized fenofibrate[9].

Particle size reduction is achieved by a variety of techniques including micronization, spray-drying, high-pressure homogenization, solvent precipitation, evaporation, supercritical fluid methods, and high-energy wet and dry milling. Although the dry milling process has often been used to improve the dissolution of fenofibrate, the wet-grinding process is comparatively rarely described in the literature[10,11]. The wet-grinding process investigated in this study was carried out using a Basket Dispersing Mill (SMA-0.75 installed a frequency changer, Shanghai, China), a piece of equipment that is widely used in the production of pigments, printing ink, food coatings, and pharmaceuticals. The mill mainly consists of four parts from the bottom up: (a) a cylindrical stainless-steel container with ground materials; (b) a centrifugal impeller driving materials into the grinding basket at a high speed; (c) a grinding basket equipped with oxidative zirconium beads used to mill materials until very fine particles are obtained; and (d) a dispersing plaque to produce dispersion, mixing, and circulation effects on materials by high-speed rotation. During the wet-grinding process, the drug is completely micronized by centrifugal, milling, and shearing forces acting simultaneously. With the combination of grinding and dispersing, the Basket Dispersing Mill is able to produce an unusual effect of reducing the particle size in short time. Furthermore, the cogrinding of fenofibrate and HPMC aqueous solution may prevent the drug particles aggregating, which usually takes place in the dry milling process.

In this study, the potential enhancement of dissolution and bioavailability of the wet-ground fenofibrate was investigated by formulating it as an immediate-release tablet. First, a ground suspension consisting of fenofibrate and HPMC aqueous solution was prepared in the Basket Dispersing Mill. Then, the suspension was granulated with the commonly used excipients and compressed into tablets. This article mainly compared the dissolution behavior and bioavailability of the wet-milled tablet and Lipanthyl® (Dijon, France), a new supra-bioavailability tablet made in France. A comparison of the dissolution between the wet-milled and the unmilled tablets was also carried out. Furthermore, optical microscopy, particle size measurement, differential scanning calorimetry (DSC), and powder X-ray diffraction (PXRD) were used to assess the state of fenofibrate in suspension.

Materials and methods

Materials

Fenofibrate was purchased from Jiangsu Nhwa Pharmaceutical Co. (Xuzhou, Jiangsu, China). Fenofibric acid and bezafibrate (internal standard) were purchased from Binhai Yujun Medicine Chemical Engineering Company (Binhai, Jiangsu, China). Polyvinylpolypyrrolidone (PVPP) and croscarmellose sodium (CCNa) were provided by ISP Technologies Inc. (Wayne, NJ, USA) and FMC Corporation Pharmaceutical Division (Philadelphia, PA, USA), respectively. Phospholipid was obtained from Shanghai Taiwei Pharmaceutical Co. (Shanghai, China). Sodium lauryl sulfate (SLS) was purchased from Hunan Erkang Pharmaceutical Co. (Changsha, Hunan, China). Lactose was obtained from DMV International Co. (BA Veghel, the Netherlands). Microcrystalline cellulose (MCC), HPMC-E5, and pregelatinized starch were purchased from Huzhou Zhanwang Pharmaceutical Co. (HuZhou, ZheJiang, China). Commercial tablets of Lipanthyl® (160 mg), used as a reference, were purchased from Laboratoires Fournier SA (H20060153, Chenove, France). All the other reagents were either of analytical or of chromatographic grade.

Methods

Determination of equilibrium solubility

The solubility of fenofibrate was determined in water and SLS solutions at various concentrations, including 0.01, 0.015, 0.02, and 0.025 mol/L, using a standardized shake flask method at 37°C. After shaking for 72 hours, the supernatant was then passed through a 0.45-μm cellulose acetate-type membrane filter, diluted with medium, and assayed by high-performance liquid chromatography (HPLC). The system conditions of HPLC detection were later described in HPLC analysis.

Preparation of wet-milled suspensions

In this process, 600 g fenofibrate was added to a cylindrical stainless-steel container filled with 500 mL of different concentrations of HPMC (with viscosity of 5 Pa S) aqueous solution: 5%, 8%, 10%, and 15% (m/v). The fenofibrate suspensions were ground in the Basket Dispersing Mill under the same controlled condition of $3188 \times g$/min for 3 hours with periodical monitoring of the particle size, and finally removed from the container. The formulations of the fenofibrate suspension are shown in Table 1.

Preparation of unmilled suspension

The unmilled suspension was prepared by adding 600 g fenofibrate to 500 mL HPMC (10%, m/v) aqueous solution and physically stirring with a glass rod until a uniform suspension was obtained.

Optical microscopy

Optical micrographs of wet-milled suspension (S3) and unmilled suspension were generated using a XSZ-G type light microscope (Chongqing Optical Instrument Co., Chongqing, China).

Particle size measurement

The particle size distribution was determined by a Laser materials Path Analyzer LS230 (Coulter Instruments, Brea, CA, USA) with evaluation of the data by Coulter LS software version 3.19. The samples tested were suspensions under different grinding time including 0, 1, 2, 3, and 4 hours, and they were diluted with the saturated solution of fenofibrate before detection. The particles of fenofibrate were sufficiently dispersed in the suspension during the process of determination.

Differential scanning calorimetry

The samples of milled and unmilled fenofibrate suspensions (10% HPMC) for DSC analysis were dried at 40°C in an oven, and then ground into powder using a mortar. The powder of HPMC was also detected as a control group. DSC analysis was carried out using a Thermal Anacyzer-60WS, DSC-60 (Shimadzu, Japan). For this, 2-mg samples were weighed in aluminum pans and analyzed with a heating rate of 10°C/min in an atmosphere of nitrogen over the temperature range of 20–200°C.

Table 1. Formulations of fenofibrate suspension.

Formulation no.	Fenofibrate (g)	HPMC aqueous solution (mL)
S1	600	5% (m/v), 500
S2	600	8% (m/v), 500
S3	600	10% (m/v), 500
S4	600	15% (m/v), 500

Powder X-ray diffraction

PXRD was used to assess the physical state of fenofibrate in unmilled and wet-milled suspensions. The samples tested were HPMC and dried powder of fenofibrate suspensions. PXRD was performed using a D/Max-2400 X-ray Fluorescence Spectrometer (Rigaku, Osaka, Japan) with a Cu Ka line as the source of radiation, and standard runs using a voltage of 56 kV, a current of 182 mA, and a scanning rate of 2°/min over a 2-theta from 3° to 45° were carried out.

Wet granulation and tableting

The prescription consisted of fillers including lactose, pregelatinized starch, and MCC, and disintegrating agents consisting of PVPP and CCNa. In addition, SLS as solubilizer and phospholipid were also added. The composition of the tablets is listed in Table 2.

The tablets were compressed with two different kinds of granules: medicated granules and blank granules prepared with MCC, with a weight ratio of 2:1. The medicated granules were prepared as follows: first of all, phospholipid was dissolved in ethanol and added to the fenofibrate suspension. Then, lactose, pregelatinized starch, PVPP, and SLS were weighed according to the formulations (Table 2) and blended in a polyethylene bag for 10 minutes to obtain a homogenous mixture, which was then added to the suspension (wet-milled or unmilled) to give a damp mass. Finally, the wet mass was passed through a 30-mesh screen and dried at 40°C in an oven for 12 hours. After drying, the granules were sized by passing them through a 24-mesh screen. The MCC granules were prepared with water by passing them through a 30-mesh screen, then dried at 40°C for 12 hours and passed through a 24-mesh screen, before final mixing with the medicated granules and CCNa before tablet formation.

A single punch tableting machine thermal design power (TDP, Shanghai, China) equipped with a capsule-type punch was used to prepare tablets with an average weight of 600 mg and at a rate of 50 tablets per minute. Each tablet contained 160 mg fenofibrate.

Dissolution experiments

The dissolution of the tablet formulations and Lipanthyl® was investigated in a ZRS-8G dissolution apparatus (Tianjin, China) according to the calibrated USP XXVIII apparatus II (paddle method). The paddle speed was 75 rpm and all experiments were carried out in triplicate. The temperature was kept at 37°C and the volume of the dissolution medium was 1000 mL. The dissolution medium was 0.025 mol/L SLS solution. In addition, tablets of F8 and Lipanthyl® were also tested in other media: water, 0.01, 0.015, and 0.02 mol/L SLS solutions. In addition, 4 mL samples were collected and replaced

Table 2. Formulations of tablets (mg/tablet).

Formulation no.	Suspension (containing fenofibrate 160 mg)	Phospholipid	SLS	Lactose	PVPP	Pregelatinized starch	MCC (granule)	CCNa
F1	S1	6	20	—	30	150	192	30
F2	S2	6	20	—	30	150	192	30
F3	S4	6	20	—	30	150	192	30
F4	S3	6	20	—	30	150	192	30
F5	S3	6	20	30	30	120	192	30
F6	S3	6	20	60	30	90	192	30
F7	S3	6	20	—	60	120	192	30
F8	S3	6	20	30	60	90	192	30
F9	S3	6	20	60	60	60	192	30
F10	S3	6	20	—	90	90	192	30
F11	S3	6	20	30	90	60	192	30
F12	S3	6	20	60	90	30	192	30
F13	S3[a]	6	20	30	60	90	192	30

[a]The unmilled suspension of S3.

by fresh medium at 5, 10, 15, 20, 30, 45 minutes. Each sample was passed through a 0.45-μm cellulose acetate-type membrane filter, diluted with the medium, and assayed by UV spectrophotometry (UV-7504, UV/Vis spectrophotometer, Xinmao Instrument Company, Shanghai, China) at 290 nm. In all experiments, the absorbance of the excipients at 290 nm was negligible.

Stability study

The tablets of F8 enclosed in foilpac were kept under closed vial conditions in a Drug Stability Test Chamber [LRH-150(250)-Y, Guangdong, China] at 40°C and 75% relative humidity (RH) for 3 months. The dissolution behavior of the tablets was evaluated in triplicate.

HPLC analysis

The equilibrium solubility of fenofibrate and the content of fenofibrate tablets were analyzed according to the US Pharmacopoeia (USP30-NF25) by HPLC using a Jasco PU-2080 Intelligent pump (Tokyo, Japan) and a Jasco UV-2075 Intelligent Detector (Tokyo, Japan). Chromatography was performed on an KYA high quality (HiQ) sil C_{18} column (4.6 × 250 mm, Japan) at room temperature using a mobile phase of 30% pure water adjusted to pH 2.5 with phosphoric acid and 70% of acetonitrile at a flow rate of 1 mL/min. The detection wavelength was set at 286 nm. These conditions resulted in a typical elution time for fenofibrate of 22 minutes.

Bioavailability study

Animals and dosing. Six beagle dogs weighing 10 ± 1 kg, raised in the Experimental Animal Center of Shenyang Pharmaceutical University, were divided into two groups and a single-dose, randomized, crossover, own-control study was carried out with a washout period of 7 days. Guidelines for experiments involving the use of animals issued by the Ethical Committee of Shenyang Pharmaceutical University were strictly followed.

After fasting overnight, dogs in groups 1 and 2 received a tablet of F8 and Lipanthyl® (160 mg, expressed as fenofibrate equivalents) with 100 mL water. Blood samples of about 2 mL were collected from a hind leg vein and transferred to heparinized tubes at 0, 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 10, 12, 24, 36, 48, 60, and 72 hours after administration. Blood samples were centrifuged at 4038 × *g* for 10 minutes using a high-speed centrifuge (TGL-16B, Shanghai, China) and plasma samples were collected and stored at –20°C.

UPLC–MS/MS analysis. In this study, fenofibric acid was determined by an ultra-performance liquid chromatography (UPLC)–tandem mass spectrometer (MS/MS) method.

The Waters ACQUITY™ UPLC system (Waters Corp., Milford, MA, USA), equipped with a binary pump system coupled to an ACQUITY™ TQD triple quadrupole tandem mass spectrometer (Waters Corp., Manchester, UK), with an electrospray ionization (ESI) source was used. Chromatographic separation was performed at 35°C using an ACQUITY BEH C_{18} column (50 × 2.1 mm ID, 1.7 μm; Waters Corp.). The following gradient was used: solvent A, 0.1% formic acid; solvent B, acetonitrile. Gradient elution was carried out as follows: 0 minute, 80% A; 0–0.7 minute, linear from 80% to 20% A; 0.7–2.0 minutes, holding at 20% for 1.3 minutes and then immediately increasing to 80% A at 2.5 minutes; 2.5–3.0 minutes, initial conditions (i.e., 80% A) for equilibration of the column. The flow rate was kept constant at 0.2 mL/min during the analysis and the sample volume injected was 5 μL. The retention times of fenofibric

acid and internal standard were 1.88 and 1.62 minutes, respectively.

The Waters ACQUITY^{TM} TQD triple quadrupole tandem mass spectrometer (Waters Corp.) was connected to the UPLC system through an ESI interface. The ESI source was operated in positive ionization mode with the capillary voltage set at 3.0 kV. The extractor and radio-frequency (RF) voltages were 3.0 and 0.1 V, respectively. The temperature of the source and desolvation was set at 100°C and 400°C, respectively. Nitrogen was used as the desolvation gas (500 L/h) and cone gas (50 L/h). For collision-induced dissociation, argon was used as the collision gas at a flow rate of 0.20 mL/min (~2.81×10^{-3} mbar). The multiple reaction monitoring mode was used for quantification. Transition reactions of fenofibric acid and internal standard were 318.9→232.9 and 362.0→315.9, respectively. All data collected in centroid mode were acquired using MasslynxTMNT4.1 software (Waters Corp.). Post-acquisition quantitative analyses were performed using a QuanLynx^{TM} program (Waters Corp.).

Fenofibric acid in dog plasma was removed by liquid–liquid extraction. To 0.2 mL dog plasma, 0.2 mL of 1 mol/L hydrochloric acid was added, followed by 20 µL internal standard solution (benzafibrate, 1.75 µg/mL in methanol). After vortex mixing for 3 minutes, 3 mL anhydrous diethyl ether was added and vortexed for 10 minutes. After centrifugation at $4250 \times g$/min for 10 minutes, the organic layer was transferred to another tube and evaporated at 40°C using a Centrifugal Concentrator (CentriVap®78120-03, Labconco Corp., Kansas City, MO, USA). The residue was dissolved in methanol and centrifuged at $12750 \times g$/min for 10 minutes then 5 µL supernatant was subjected to UPLC–MS/MS analysis. Quantification was based on the peak area ratio R (area of fenofibric acid/area of internal standard (AFA/AIS)). Linearity was observed over the concentration range of 10.43–10,430 ng/mL with correlation coefficients of over 0.99. A typical calibration curve was as follows: $R = 5.6454C + 34.4315$($r = 0.9974$, $n = 9$). The lower limit of quantification for the determination of fenofibric acid in dog plasma was 10.43 ng/mL. The accuracy of the analytical method was 100.61 ± 2.25% and within-day and between-day precision was 6.89% and 6.10%, respectively. The extraction recovery of fenofibric acid in dog plasma ($n = 9$) was 94.41 ± 7.53%.

Statistical analysis. The pharmacokinetic parameters were calculated using the drug and statistics version 2.0 software (Mathematical Pharmacology Professional Committee of China, Shanghai, China). All the concentration–time curves of fenofibric acid in dog plasma fitted a two-compartment model with a weighting factor of $1/C^2$. C_{max} and T_{max} were obtained from the raw data. The area under the curve to the last measurable concentration [$AUC_{(0-t)}$] was calculated by the linear trapezoidal rule.

Results and discussion

Solubility study in different media

Fenofibrate is nearly insoluble in water with an aqueous solubility of about 0.3 µg/mL (at 37°C). Table 3 summarizes the experimentally determined solubility of fenofibrate in pure water, 0.01, 0.015, 0.02, and 0.025 mol/L SLS solutions. The results in Table 3 indicate that the solubility of fenofibrate increases along with the concentration of SLS. Thus, SLS was employed as a wetting and solubilizing agent in the dissolution medium for simulating the sink effect in the gastrointestinal tract[12].

Physical characterization of fenofibrate

The optical micrographs and particle size distribution of fenofibrate suspensions are shown in Figure 1 and Table 4, respectively. It can be seen from Figure 1 that the uniformity and particle size reduction of fenofibrate particles are substantially improved after grinding. As shown in Table 4, the particle size is obviously reduced from 0 to 3 hours after grinding; however, no significant reduction is observed between 3 and 4 hours. So considering the efficiency and energy conservation, the grinding time was set for 3 hours.

In this study, DSC and PXRD were used to confirm the crystalline status of fenofibrate in suspension, and the samples were dried powders of HPMC, wet-milled, and unmilled suspensions. Fenofibrate has a melting point reported to be in the range of 79–82°C[13]. Figure 2 shows the DSC thermograms over the temperature range 20–200°C, and both the wet-milled and the unmilled suspensions display a sharp endotherm in the DSC thermogram at about 81°C. The PXRD patterns of fenofibrate suspensions are presented in Figure 3. The identical X-ray patterns that are consistent with the DSC result confirmed that the crystals of fenofibrate

Table 3. Solubility study of fenofibrate in various media at 37°C in µg/mL (mean ± SD, $n = 3$).

Water	0.01 mol/L SLS	0.015 mol/L SLS	0.02 mol/L SLS	0.025 mol/L SLS
0.28 ± 0.1	58.3 ± 0.4	131.8 ± 0.3	182.9 ± 0.3	251.5 ± 0.1

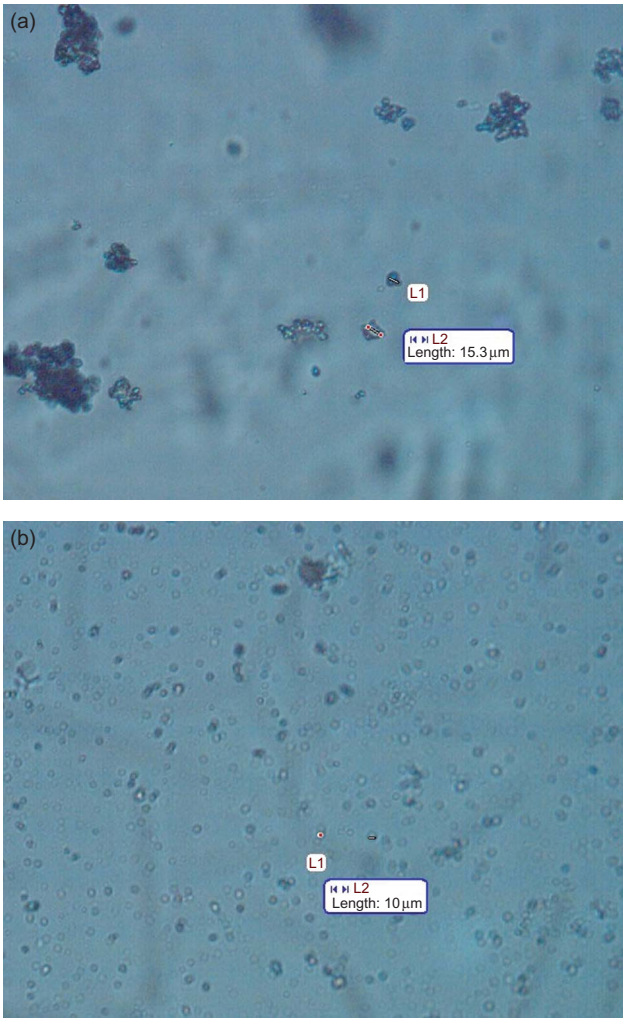

Figure 1. Optical micrographs of (a) unmilled fenofibrate suspension and (b) wet-milled fenofibrate suspension.

Table 4. Particle size distribution of fenofibrate in suspensions under different grinding time.

Grinding time (hours)	d_{10} (µm)	d_{50} (µm)	d_{90} (µm)
0	3.6	11.8	35.5
1	2.8	6.4	14.6
2	2.4	4.8	11.1
3	1.6	3.6	8.0
4	1.5	3.4	7.0

were maintained in the ground suspension, and the same conclusion was reported in the published literature[10]. So, it appears that the fenofibrate adheres to the HPMC particles with submicron crystals instead of being transformed into an amorphous form, a thermodynamically unstable state tends to convert to the stable, crystalline state[14].

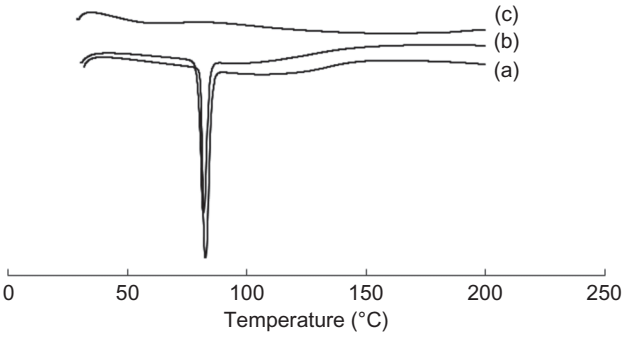

Figure 2. DSC thermograms: (a) dried powder of unmilled suspension, (b) dried powder of wet-milled suspension, and (c) HPMC.

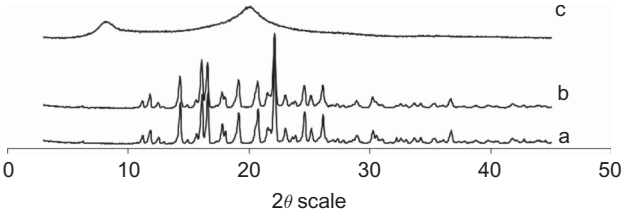

Figure 3. Powder X-ray powder diffraction patterns of (a) unmilled suspension, (b) wet-milled suspension, and (c) HPMC.

In vitro dissolution

Dissolution study of tablets prepared with different suspensions

Figure 4 compares the dissolution behavior of tablets prepared with 5%, 8%, 10%, and 15% HPMC suspensions. During the suspension, tablets of S3 (10% HPMC) displayed an excellent dissolution profile with 90% being dissolved in 30 minutes, whereas that of S1 (5% HPMC), S2 (8% HPMC), and S4 (15% HPMC) exhibited dissolution of 68%, 78%, and 79%, respectively. The

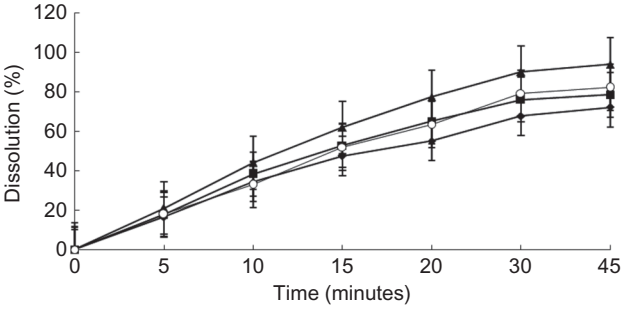

Figure 4. Dissolution profiles of tablets prepared with different concentrations of HPMC in 0.025 mol/L SLS (bars donate standard error (SE)). (◆) 5% HPMC; (■) 8% HPMC; (▲) 10% HPMC; and (○) 15% HPMC.

results show that the improved dissolution is not linear with the increase of HPMC, which should be maintained at a certain level. In the formulation, HPMC acts as a hydrophilic carrier allowing adhesion of the drug particles. Therefore, the wettability and stability of fenofibrate are improved by the carrier, and sometimes the carrier dissolution dictates the drug release profile[4]. Lower dissolution is induced by too little HPMC carriers, however, if too much is present, the increased amount of polymer around tablets leads to gelation which inhibits the release of effective substance[15]. So, the polymer (HPMC) concentration was considered to be a decisive factor in controlling drug release rate[16] and only at a certain concentration (10% in this approach) can fenofibrate exhibit better dissolution behavior.

Dissolution from developed tablet formulations

Although the dissolution was 90% within 30 minutes, the fenofibrate in the F4 was not released completely. Therefore, the dissolution behavior of the developed tablet formulations with different amounts of various excipients was investigated in this study. The dissolution profiles are illustrated in Figure 5. From F4 to F12, the effect of the amount of PVPP on the dissolution behavior of tablets was evaluated. The dissolution rate of F4–F6, F7–F9, and F10–F12 was 90–96%, 93–98%, and 86–87% in 30 minutes, respectively. These results indicated that the improved dissolution did not increase along with PVPP. This is mainly because of the fact that moderate PVPP accelerates tablet disintegration; however, if excessive, the hydrophobic effect produced by PVPP will be greater than the disintegration, resulting in a lower dissolution. The effect of lactose on dissolution behavior was also investigated. The data showed that the dissolution rate increased in the presence of lactose because of the hydrophilic lactose on the fenofibrate surface, which enabled more effective wetting of tablets. In addition, it is hypothesized that the wettability of the small lipophilic fenofibrate particles is also improved by the presence of the surfactant SLS[10]. Among all the formulations, F8 exhibited the best drug dissolution and was finally chosen as the prescription for the fenofibrate tablet.

The wet-ground tablet of F8 was also compared with the Lipanthyl® tablet, the unmilled tablet of F13, and the tablet of F8 stored for 3 months at 40°C and 75% RH. As can be seen in Figure 6, the dissolution of the wet-ground tablet was 98% in 30 minutes, slightly higher than for the Lipanthyl® tablet (92% in 30 minutes). However, this was significantly higher than that of the unmilled tablets (56% in 30 minutes). Dissolution rate enhancement of fenofibrate is often caused by production of the amorphous state. However, according to the DSC and PXRD analyses, the improved dissolution of the wet-milled tablet may be because of the generation of submicron drug crystals, and these crystals were sufficiently small (i.e., nanoscale) to offer a large surface area as well as an increased saturation concentration (Kelvin law) and, hence, a high dissolution rate[17]. Furthermore, no significant decrease in the dissolution rate (96% in 30 minutes) of F8 was observed after 3 months of storage. For the stabilization of fenofibrate in a system where hydrogen-bonding interactions are not possible, it may be due to the binding of subcrystalline fenofibrate to HPMC carrier, which would reduce drug mobility and, hence, prevent the drug aggregating during storage.

During this research, it was found that the dissolution rate of tablets was limited by the disintegration to a large extent. Many methods were tried to solve the problem of tablet disintegration. For example, although various disintegrants were added when preparing the granulation or the finished granulation before tablet formation, or both, the tablets did not collapse completely within 30 minutes. The disintegration of tablets

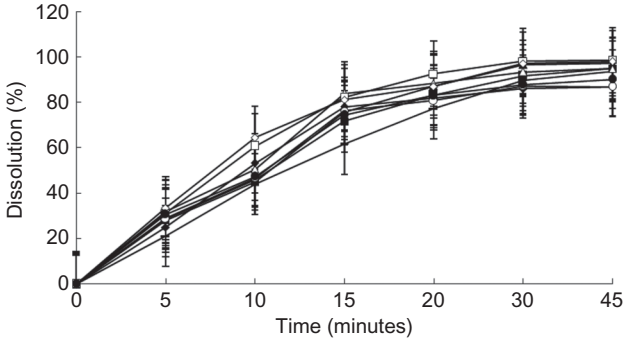

Figure 5. Fenofibrate dissolution of different formulations in 0.025 mol/L SLS (bars donate SE). (–) F4; (■) F5; (▲) F6; (△) F7; (□) F8; (◇) F9; (◆) F10; (○) F11; and (●) F12.

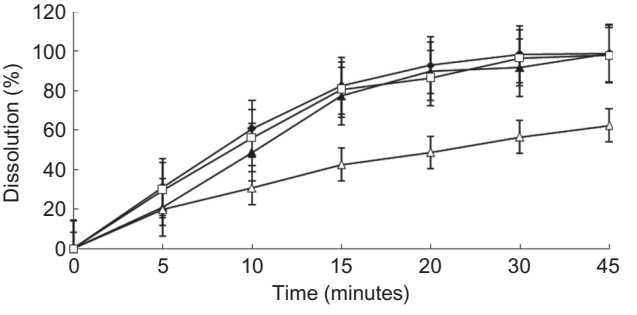

Figure 6. Dissolution profiles of different fenofibrate tablets in 0.025 mol/L SLS (bars donate SE). (◆) F8 of wet-milled tablets; (▲) Lipanthyl® tablets; (△) F13 of unmilled tablets; and (□) F8 stored at 40°C, 75% RH for 3 months.

could be significantly improved by adding an effervescent agent to the formulations; however, the tablets displayed poor stability due to the high hygroscopicity of the effervescent agent. As the main reason for the disintegration problem was the viscosity generated by the high concentration of HPMC, blank MCC granules were mixed with the drug granules before tableting to reduce the viscosity and then shorten the disintegration time.

Dissolution of fenofibrate tablets in different media

The dissolution behaviors of F8 and Lipanthyl® tablets were also evaluated in other media including water, 0.01, 0.015, and 0.02 mol/L SLS solutions. As shown in Figure 7, both the wet-milled tablet and the Lipanthyl® tablet exhibited a dissolution of only 1% or less within 30 minutes in the pure water medium. The sharp decrease in the amount of dissolved drug can be ascribed to the solubility of fenofibrate in pure water[3]. Because the wettability and dissolubility of fenofibrate were improved by SLS, the saturation concentration and the dissolution of fenofibrate also increased. As can be seen in Figures 6 and 7, the wet-milled tablet displays a similar dissolution behavior to the Lipanthyl® tablet, and the absorption of the two different tablets in gastrointestinal tract will need to be investigated.

Bioavailability study

The wet-milled tablet (F8, 160 mg) chosen as the test and the commercial suprabioavailability tablet (Lipanthyl®, 160 mg) as a reference were orally administered to six beagle dogs under fasting conditions, to further investigate the oral bioavailability. Fenofibrate, a prodrug, is hydrolyzed by intestinal, plasma, and tissue esterases to the active metabolite fenofibric acid. There is essentially no unchanged fenofibrate detectable in plasma after oral administration according to the published reports[18]. Therefore, the pharmacokinetic evaluation of fenofibrate is based on the quantification of fenofibric acid in plasma[19].

The mean plasma fenofibric acid concentration versus time curves for both test and reference materials are shown in Figure 8, and the pharmacokinetic parameters are given in Table 5. From the results, it can be seen that more rapid absorption with a T_{max} of 2.63 hours was obtained for the test compared with the reference with a T_{max} of 3.75 hours ($P > 0.05$). The mean peak concentration of the F8 tablet (4.63 ± 1.71 μg/mL) was doubled to that of the Lipanthyl® tablet (2.11 ± 0.08 μg/mL) ($P < 0.05$). As is shown in Figure 8, the area under the plasma concentration–time curve of the test [$AUC_{(0-t)}$ = 46.83 ± 11.09 μg/mL·h] was obviously higher than the reference [$AUC_{(0-t)}$ = 35.12 ± 10.97 μg/mL·h]. Upon normalization with the $AUC_{(0-t)}$ value of Lipanthyl® tablet, the bioavailability of the wet-milled tablet was approximately 1.3-fold higher.

In the in vivo study, the bioavailability of the wet-milled tablet was higher than that of the suprabioavailability Lipanthyl® tablet, which was not consistent with the dissolution rate in the in vitro experiment. In addition to the reduced particles, the improved bioavailability may also be due to the SLS and phospholipid

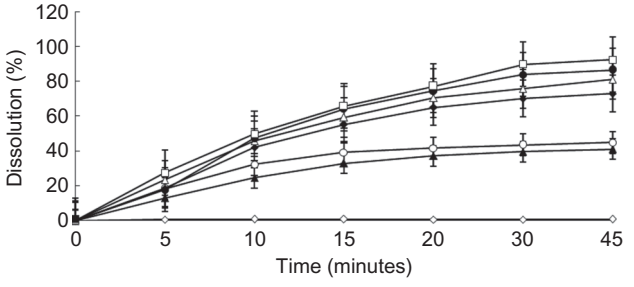

Figure 7. Dissolution profiles of fenofibrate from F8 and Lipanthyl® in different dissolution media (bars donate SE). (✧) Lipanthyl® in pure water; (■) F8 in pure water; (▲) Lipanthyl® in 0.01 mol/L SLS; (○) F8 in 0.01 mol/L SLS; (◆) Lipanthyl® in 0.015 mol/L SLS; (△) F8 in 0.015 mol/L SLS; (●) Lipanthyl® in 0.02 mol/L SLS; and (□) F8 in 0.02 mol/L SLS.

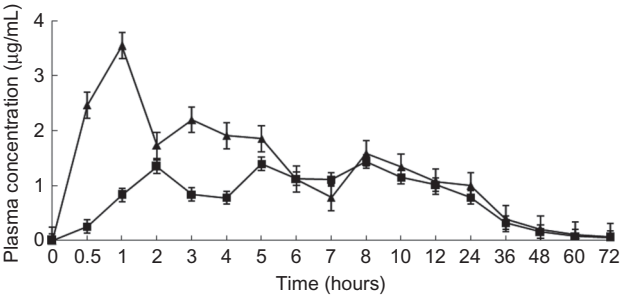

Figure 8. Mean fenofibric acid plasma profiles after oral administration of wet-milled tablet (▲) and reference Lipanthyl® tablet (■) (n = 6; bars donate SE).

Table 5. Pharmacokinetic parameters after oral administration of wet-milled tablet and Lipanthyl® tablet.

Formulation	T_{max} (hours)	C_{max} (μg/mL)	$T_{1/2}$ (hours)	$AUC_{(0-t)}$ (μg/mL·h)	$AUC_{(0-\infty)}$ (μg/mL·h)	Relative Bioavailability (%)[a]
Wet-milled tablet	2.63	4.63 ± 1.71	11.60 ± 2.38	46.83 ± 11.09	47.69 ± 11.65	133.34
Lipanthyl® tablet	3.75	2.11 ± 0.08	16.01 ± 9.69	35.12 ± 10.97	36.12 ± 11.03	

[a]Calculated from $AUC_{(0-t)}$ with Lipanthyl® tablet as reference.

added to the test tablet. The saturation solubility and dissolution of fenofibrate improved in the presence of the surfactant SLS and, hence, the absorption in the gastrointestinal tract was increased. Furthermore, the phospholipid contained in the formulation could improve the bioavailability because of its physicochemical structure.

An unexpected finding not described elsewhere in the literature was the multiple peaks in both plasma concentration–time curves. There are several reasons to explain this novel phenomenon. The second peak can be explained by the fact that a large amount of fenofibrate (160 mg) was administered; however, as the preparation stayed in the stomach for only about 30 minutes in the beagle dogs, it was impossible for fenofibrate to dissolve completely within such a short time, and it was not fully absorbed. About 30 minutes after drug administration, the preparation began to enter the intestinal tract, and dissolution and absorption continued. In addition, the possible role of gastrointestinal motility as a determinant of the phenomena of secondary maxima occurring only in the fasted state has been previously addressed. Such a possibility is quite reasonable because gastrointestinal motility patterns are unique in the fasted state in humans and dogs and is altered drastically in the fed state in the two species[20]. Furthermore, according to Yves Plusquellec et al., if the assumption of enterohepatic recirculation is rejected by physiological arguments, a possible explanation for the occurrence of multiple peaks may be a discontinuous absorption along the gut[21]. Fenofibrate absorption of different sites in the gastrointestinal tract was also reported in the literature[9]. So it is hypothesized that the third concentration peak may be caused by the discontinuous absorption of undissolved drug in the gastrointestinal tract.

Table 5 shows a faster absorption and higher mean peak concentration of the test compared with the reference. In addition, as seen in Figure 8, the three concentration peaks of the test decrease one by one, whereas those of the reference are similar. This is possible because of the difference in their drug release behavior. Although the Lipanthyl® tablet exhibited gradual release during the dissolution process, the wet-milled tablet dissolved quickly at first but then slowed down mainly because of the HPMC gel layer formed on the surface of the tablet[15]. The novel phenomenon of multiple peaks was obtained mainly resulting from the feature of the preparations or absorption characteristics in the gastrointestinal tract of the beagle dog.

Conclusion

This study shows that both the dissolution in vitro and the oral bioavailability of fenofibrate could be obviously improved by grinding with the hydrophilic carrier HPMC, higher than the unmilled and commercial suprabioavailability Lipanthyl® tablet. Particle size reduction was successfully obtained in the Basket Dispersing Mill without changing the crystal form into an amorphous one, according to the particle size, DSC, and PXRD analyses results. The recrystallization and mobility of the fenofibrate particles were also prevented during storage by cogrinding with HPMC. Therefore, the wet-grinding method is a powerful technique to enhance the dissolution and bioavailability of poorly water-soluble drugs, especially for BCS Class II compounds.

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Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this paper.

References

1. Murali Mohan Babu GV, Prasad CDS, Ramana Murthy KV. (2002). Evaluation of modified gum karaya as carrier for the dissolution enhancement of poorly water-soluble drug nimodipine. Int J Pharm, 234:1–17.
2. Jinno J, Kamada N, Miyake M, Yamada K, Mukai T, Odomi M, et al. (2008). In vitro–in vivo correlation for wet-milled tablet of poorly water-soluble cilostazol. J Control Release, 130:29–37.
3. Waard H, Hinrichs WLJ, Visser MR, Bologna C, Frijlink HW. (2008). Unexpected differences in dissolution behavior of tablets prepared from solid dispersions with a surfactant physically mixed or incorporated. Int J Pharm, 349:66–73.
4. Vasconcelos T, Sarmento B, Costa P. (2007). Solid dispersions as strategy to improve oral bioavailability of poor water soluble drugs. Drug Discov Today, 12:23–4.
5. Jain RA, Brito L, Straub JA, Tessier T, Bernstein H. (2008). Effect of powder processing on performance of fenofibrate formulations. Eur J Pharm Biopharm, 69:727–34.
6. Sanganwar GP, Gupta RB. (2008). Dissolution-rate enhancement of fenofibrate by adsorption onto silica using supercritical carbon dioxide. Int J Pharm, 360:213–8.
7. Wishart DS, Konx C, Guo AC, Shrivastava S, Hassanali M, Stothard P, Chang Z, Woolsey J. (2006). Drug bank: A comprehensive resource for in silico drug discovery and exploration. Nucleic Acids Res, 1:D668-D672.
8. Sant VP, Smith D, Leroux JC. (2005). Enhancement of oral bioavailability of poorly water-soluble drugs by poly (ethylene glycol)-block-poly (alkyl acrylate-co-methacrylic acid) self-assemblies. J Control Release, 104:289–300.
9. Hanafy A, Spahn-Langguth H, Vergnault G, Grenier P, Tubic Grozdanis M, Lenhardt T, et al. (2007). Pharmacokinetic evaluation of oral fenofibrate nanosuspensions and SLN in comparison to conventional suspensions of micronized drug. Adv Drug Deliv Rev, 59:419–26.

10. Vogt M, Kunath K, Dressman JB. (2008). Dissolution enhancement of fenofibrate by micronization, cogrinding and spray-drying: Comparison with commercial preparations. Eur J Pharm Biopharm, 68:283–8.
11. Vogt M, Vertzoni M, Kunath K, Reppas C, Dressman JB. (2008). Cogrinding enhances the oral bioavailability of EMD 57033, a poorly water soluble drug, in dogs. Eur J Pharm Biopharm, 68:338–45.
12. Tan A, Simovic S, Davey AK, Rades T, Prestidge CA. (2009). Silica-lipid hybrid (SLH) microcapsules: A novel oral delivery system for poorly soluble drugs. J Control Release, 134:62–70.
13. Huang QP, Wang JX, Zhang ZB, Shen ZG, Chen JF, Yunb J. (2009). Preparation of ultrafine fenofibrate powder by solidification process from emulsion. Int J Pharm, 368:160–4.
14. Brodka-Pfeiffer K, Häusler H, Grass P, Langguth P. (2003). Conditioning following powder micronization: Influence on particle growth of salbutamol sulfate. Drug Dev Ind Pharm, 29(10):1077–84.
15. Savaşer A, Özkan Y, Işimer A. (2005). Preparation and in vitro evaluation of sustained release tablet formulations of diclofenac sodium. Farmaco, 60:171–7.
16. Khan GM, Meidan VM. (2007). Drug release kinetics from tablet matrices based upon ethylcellulose ether-derivatives: A comparison between different formulations. Drug Dev Ind Pharm, 33:627–39.
17. Waard H, Hinrichs WLJ, Frijlink HW. (2008). A novel bottom–up process to produce drug nanocrystals: Controlled crystallization during freeze-drying. J Control Release, 128:179–83.
18. Mertens B, Cahay B, Klinkenberg R, Streel B. (2008). An automated method for the simultaneous determination of pravastatin, 3-hydroxy isomeric metabolite, pravalactone and fenofibric acid in human plasma by sensitive liquid chromatography combined with diode array and tandem mass spectrometry detection. J Chromatogr A, 1189:493–502.
19. Chen YP, Lu Y, Chen JM, Lai J, Sun J, Hu FQ, et al. (2009). Enhanced bioavailability of the poorly water-soluble drug fenofibrate by using liposomes containing a bile salt. Int J Pharm, 376:153–60.
20. Takamatsu N, Welage LS, Hayashi Y, Yamamoto R, Barnett JL, Shah VP, et al. (2002). Variability in cimetidine absorption and plasma double peaks following oral administration in the fasted state in humans: Correlation with antral gastric motility. Eur J Pharm Biopharm, 53:37–47.
21. Plusquellec Y, Efthymiopoulos C, Duthil P, Houin G. (1999). A pharmacokinetic model for multiple sites discontinuous gastrointestinal absorption. Med Eng Phys, 21:525–32.