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ORIGINAL ARTICLE

Comparative *In Vitro* and *In Vivo* Evaluation of Fenofibric Acid as an Antihyperlipidemic Drug

Antihiperlipidemik İlaç Olarak Fenofibrik Asidin Karşılaştırmalı *İn Vitro* ve *İn Vivo* Değerlendirilmesi

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ABSTRACT

Objectives: Fenofibric acid (FA) is antihyperlipidemic agent and commercially available as a tablet formulation that weighs 840 mg for 105 mg of active substance. A new formulation with less inactive substance was developed as an alternative to the conventional formulation. The purpose of this study was to evaluate the dissolution and the relative bioavailability of the surface solid dispersion (SSD) and conventional formulations of FA by comparing them with the reference formulation in its commercial tablets. The *in vitro-in vivo* correlation among these tablet formulations was also evaluated.

Materials and Methods: The dissolution study was performed in phosphate buffer pH 6.8 and biorelevant fasted state simulated intestinal fluid. Dissolution efficiency and mean dissolution time (MDT) were used to compare the dissolution profiles. The bioavailability study, using nine healthy volunteers, was conducted based on a single-dose, fasted, randomized, crossover design. The *in vivo* performance was compared using the pharmacokinetic parameters C_{max}, T_{max}, AUC_{0-72}, and $AUC_{0-\infty}$. A linear correlation model was tested using MDT and mean residence time (MRT).

Results: The results indicated that there were significant differences in the dissolution performances but no significant differences among the mean C_{max}, T_{max}, AUC_{0-72}, or $AUC_{0-\infty}$ estimated from the SSD, conventional, and reference formulations. A poor correlation was found between MRT and MDT of the three formulations.

Conclusion: The SSD formulation led to an instantaneous dissolution of the drug due to the presence of the polymer and the physical structure of the SSD. The conventional formulation could not achieve rapid dissolution despite its satisfying the requirement for immediate drug release dosage form. Both formulations could be considered bioequivalent with the reference. The *in vitro* dissolution behavior of FA using a single medium did not reflect their *in vivo* properties in the fasted condition. There was no correlation between the *in vitro* dissolution and the in vivo bioavailability of FA in this condition.

Key words: Fenofibric acid, surface solid dispersion, dissolution, bioavailability, correlation

ÖZ

Amaç: Fenofibrik asit (FA) antihiperlipidemik bir ajandır ve 105 mg aktif madde içeren 840 mg ağırlığında bir tablet formülasyonu şeklinde ticari olarak temin edilebilir. Konvansiyonel formülasyona alternatif olarak inaktif madde miktarı daha az yeni bir formülasyon geliştirildi. Bu çalışmanın amacı, yüzey katı dispersiyonunun (SSD) ve konvansiyonel FA formülasyonlarının disolüsyonunu ve göreceli biyoyararlanımını ticari tabletlerindeki referans formülasyon ile karşılaştırmaktır. Bu tablet formülasyonları arasındaki *in vitro-in vivo* korelasyon da değerlendirildi.

Gereç ve Yöntemler: Disolüsyon deneyleri fosfat tamponu pH 6,8 ve açlık durumu yapay bağırsak sıvısı içinde yapıldı. Disolüsyon profillerini karşılaştırmak için disolüsyon verimliliği ve ortalama disolüsyon süresi (MDT) kullanıldı. Dokuz sağlıklı gönüllü üzerinde gerçekleştirilen biyoyararlanım çalışması, tek doz, aç karna, randomize, çapraz bir tasarım kullanılarak gerçekleştirildi. *In vivo* performans, C_{max}, T_{max}, AUC_{0-72} ve $AUC_{0-\infty}$ farmakokinetik parametreleri kullanılarak karşılaştırıldı. MDT ve ortalama kalış süresi (MRT) kullanılarak doğrusal korelasyon modeli test edilmiştir.

Bulgular: Sonuçlar, çözünme performanslarında önemli farklılıklar olduğunu, ancak SSD, geleneksel ve referans formülasyonlardan tahmin edilen ortalama C_{max}, T_{max}, AUC_{0-72} veya $AUC_{0-\infty}$ arasında önemli farklılıklar olmadığını gösterdi. Üç formülasyonun MRT ve MDT değerleri arasında zayıf bir korelasyon bulundu.

Sonuç: SSD formülasyonu, polimerin varlığı ve SSD'nin fiziki yapısı nedeniyle ilacın ani disolüsyonuna yol açtı. Konvansiyonel formülasyon, hızlı salım dozaj formu gereksinimini karşılamasına rağmen disolüsyonu hızlı olmadı. Her iki formülasyon da referansın biyoeşdeğeri olarak değerlendirilebilir.

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FA'nın bir ortamdaki *in vitro* disolüsyon profili, açlık durumunda *in vivo* özelliklerini yansıtmamıştır. Bu durumda fenofibrik asidin *in vitro* disolüsyonu ile *in vivo* biyoyararlanımı arasında korelasyon yoktu.

Anahtar kelimeler: Fenofibrik asit, yüzey katı dispersiyonu, disolüsyon, biyoyararlanım, korelasyon

INTRODUCTION

Fenofibric acid (FA), the active moiety of fenofibrate, is an antihyperlipidemic agent because it is the synthetic ligand that binds to nuclear peroxisome proliferator-activated receptors alpha.[1-3] FA is a carboxylic acid moiety, while fenofibrate is an ester moiety.[4] Figure 1 shows the chemical structures of both FA and fenofibrate. In its marketed form, fenofibrate is insoluble and recommended to be taken with food, and it typically includes nonmicronized tablets, micronized capsules, microcoated micronized tablets, and hard gelatin capsules. The nanocrystal formulation of fenofibrate and the conventional formulation of FA currently available on the market can be taken with or without food.[3] A single 105 mg dose of FA is bioequivalent to a single 145 mg dose of fenofibrate in both fed and fasted states.[5] Not only is the production of the nanocrystal formulation of fenofibrate inflexible, but the high cost also has to be taken into account. As a result, FA has been chosen and developed as an alternative to fenofibrate for oral administration.

Like fenofibrate, FA is mainly absorbed from the gastrointestinal (GI) tract. However, it does better than fenofibrate, causing its bioavailability to be higher than that of fenofibrate in all GI regions.[6] The absolute oral bioavailability of FA in rats stands at 40%.[7] Physicochemically, FA is characterized as a poorly soluble weak acid drug. The pKa of FA is 4 and the log P is 3.85.[8] FA has relatively poor solubility at gastric pH (the pH is lower than its pKa), but it has fairly good solubility at intestinal pH.[1] The solubility of FA is 162.5 µg/mL in water and 1156 µg/mL at pH 6.8.[9] Due to its adequate permeability, FA is classified as a class II drug in the Biopharmaceutical Classification system (BCS) subclass (a) for weak acids.[10] The poor solubility of FA in water may cause its dissolution to be reasonably slow and its bioavailability to be unpredictable.

Recently, FA has become commercially available as a tablet formulation, namely Fibricor® (the brand for 105 mg FA). The weight of this formulation is 840 mg and it consists of many ingredients for the active substance of 105 mg. The dosage form of FA with increased dissolution is developed to examine other possible platforms. The dissolution rate of BCS class II drugs is the limiting step for their oral bioavailability. The surface solid dispersion (SSD) formulation is regarded as a method to improve the dissolution rate and bioavailability of poorly soluble drugs. The distribution of drug particles on the carrier surface can enhance the wettability, dissolution rate, and consequently bioavailability of drugs.[11,12] The FA SSD has been investigated. In the simulated intestinal fluid, the data showed that the dissolution of FA increased more than that of the pure drug.[13] In the present study, a new FA formulation with enhanced dissolution and less inactive ingredients was developed and evaluated for its *in vitro* and *in vivo* performance. It has never appeared in any publication.

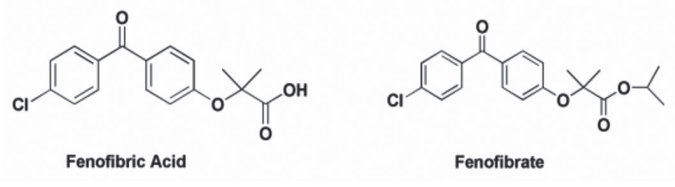

Figure 1. Chemical structures of fenofibric acid and fenofibrate

The drug dissolution rate and bioavailability are influenced by the manufacturing process and the changes happening during formulation. Therefore, bioavailability issues are frequently used to assess the safety and efficacy of drug products. Only two studies have been reported so far to enhance the dissolution and bioavailability of FA. The FA loaded pellet is prepared with magnesium carbonate and k-carrageenan employing the extrusion/spheronizing technique followed by coating with ethyl cellulose. The pellet is bioequivalent to the commercial product in beagle dogs.[14] Additionally, a mixture of FA and magnesium carbonate at a weight ratio of 2/1 can improve the solubility, dissolution, and oral bioavailability of FA.[9] No information about the *in vitro-in vivo* correlation (IVIVC) of this drug is available. One of the challenges of biopharmaceutics research is to figure out the correlation of the *in vitro* drug release information of various drug formulations with the *in vivo* drug profiles. In relation to FA, the correlation between the dissolution rate and the *in vivo* performance is likely to be predicted.

The present study examined the results of both the bioavailability and the dissolution of the two tested formulations and the immediate-release reference formulation. The formulations tested herein are the SSD and conventional methods. The *in vitro* dissolution characteristics of these tablets exhibited different release patterns, meaning the correlation between the *in vitro* dissolution and the *in vivo* bioavailability of these tablet formulations is also under investigation.

MATERIALS AND METHODS

Materials

The FA and the standard FA used in this study were purchased from BOC Science and Sigma, both of which are based in the USA. Other materials such as croscarmellose sodium (CS), Avicel PH 101, lactose monohydrate, Manihot starch, magnesium stearate, and talc were obtained from the local supplier in Indonesia. The 105 mg FA® tablets, the generic version of Fibricor® reference tablets (Mutual Pharmaceutical), were bought from International Pharmacy, USA. The biorelevant medium fasted state simulated intestinal fluid (FaSSIF) was purchased from Biorelevant.com Ltd (Croydon, UK). Then a number of materials were acquired from the Merck Group of

Germany, including sodium hydroxide, potassium dihydrogen phosphate, sodium dihydrogen phosphate monohydrate, and sodium chloride. In addition, the distilled water used for all dissolution experiments and all other reagents were of analytical grade. The IS of 4'-chloro-5-fluoro-2-hydroxyl benzophenone (CFHB) was obtained from Apollo Sci (UK), the blank plasma from the Indonesia Red Cross, Bandung (Indonesia), and the rest (methanol, ethyl acetate, hydrochloric acid, and acetonitrile) from JT Baker (USA). All reagents used herein were of analytical grade, with the exception of acetonitrile of high performance liquid chromatography (HPLC) grade.

Methods

Preparation of surface solid dispersion and conventional formulations

The SSD formulation of FA with CS (1:1 w/w) was prepared by the solvent evaporation method. First, the drug was dissolved in ethanol to obtain a clear solution. The carrier CS was then dispersed in the drug solution, and the solvent was removed using a rotary evaporator. The viscous residues produced were dried in an oven at 40˚C to allow complete evaporation of ethanol in order to obtain constant weight of powder. Subsequently, the mass was passed through a 40 mesh sieve to get dry free-flowing powder ready for compression into tablets by the direct compression method. Avicel PH 101 and magnesium stearate (1% w/w) were later added as a diluent and lubricant. This mixture was checked for flowability and compressibility before the compression of this mass into tablets. The blend was compressed by a single punch tablet press with punch size 10 mm into 300 mg tablets with an FA concentration of 105 mg.

The conventional formulation was prepared by the wet granulation method. The drug was mixed thoroughly with lactose monohydrate as a diluent and then granulated with starch paste (10% w/w). The dried granules were incorporated with dried starch (10% w/w), magnesium stearate (1% w/w), and talc (2% w/w). The same procedures for flowability, compressibility, and compression were also applied to this mixture with the same tablet press, punch size, and FA concentration.

Besides the above formulations, the reference formulation of FA was also used in the present study. FA® itself is actually a generic version of Fibricor®, whose formulation contains FA, copovidone, crospovidone, magnesium stearate, and microcrystalline cellulose in its 840 mg tablet weight.

Drug content uniformity in tablet formulations

In each formulation, the tablet samples were weighed accurately and transferred into a 100 mL volumetric flask. The solvent mixture of 2 M urea and 1 M sodium citrate (5 mL each) was added, and the mixture was heated for 15 min. This procedure was performed for the solubilization of FA, and the solvent mixture was used as a hydrotropic agent.[15] The solution was eventually filtered through Whatman filter paper, while the remaining filtrate was diluted with distilled water and analyzed using a ultraviolet/visible (UV/Vis) spectrophotometer (Shimadzu 1800A) at 299 nm. The FA concentration was determined based on the calibration curve previously built. The experiment for drug content was repeated three times, and the results were expressed as the mean ± standard deviation.

Dissolution studies

The release characteristics of the tested formulations and the reference formulation were evaluated for the dissolution rate in a type 2 (paddle) dissolution apparatus (Electrolab TDT-08L, USP), using 500 mL of phosphate buffer pH 6.8, and the biorelevant medium FaSSIF was maintained at 37±0.5˚C. The paddle rotation speed was set at 50, 75, and 100 rpm. The samples were taken at specified time intervals and replaced with equal volumes of fresh dissolution media to maintain constant volumes in the flasks. The samples were filtered through a 0.45 µm membrane. The filtered samples were diluted with the dissolution medium, and the FA concentration was determined by a UV/Vis spectrophotometer at the wavelengths of 298.7 nm for phosphate buffer and 299 nm for FaSSIF. The FA concentration was determined based on the calibration curve previously built. The dissolution experiment was conducted three times, and the results were expressed as the mean values of the dissolution efficiency $(DE)_{60}$ parameter (%).

Bioavailability studies

This study was approved by the Health Research Ethics Committee of the Faculty of Medicine Universitas Padjadjaran Bandung (897/UN6.C.10/PN/2017) in accordance with the Declaration of Helsinki and International Conference on Harmonisation-Good Clinical Practice guidelines. There were nine eligible subjects included in the present study. The subjects were all healthy and male. Their age varied from 22 to 48 years, weight from 47 to 68 kg, and height from 155 to 175 cm. These values give the standard body mass index 18-25 kg/m^2. Furthermore, they were required not to have any significant medical history and evidence of hepatic, renal, GI, or hematological disorders; acute or chronic diseases; clinically significant abnormalities; or drug abuse or allergy. Moreover, they were instructed to abstain from taking any concomitant medication, food supplement, or herbal medicine for at least 14 days prior to and during the study. Subjects were excluded if they had participated in any clinical study or used the investigational drugs within the past 30 days prior to starting the present study. In addition, caffeine-containing beverages were not allowed while the study was being conducted. All chosen participants were given written informed consent forms after the nature and purpose of the study were explained.

The protocol applied a randomized, three-way crossover design with nine subjects in each period. In the first period, after overnight fasting and predose blood sampling, every subject was given a single dose of any formulation in a random way along with 250 mL of water. Food and drinks (other than water 2 h after dosing) were not allowed until 4 h after dosing. Standard meals for both lunch and dinner were served at 4 and 10 h, respectively, while a snack was given 8 h after drug administration. Blood pressure, heart rate, respiration rate, and adverse events were monitored during blood sampling. Approximately 5 mL of the serial venous blood samples were drawn using 22G drawing needles into VACUETTE® tubes

containing 100 µL of sodium citrate 0.485 M as the anticoagulant predose (0 h) and at 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 8, 10, 12, 16, and 24 h postdose.[5] The blood samples were centrifuged with Germany's EBA 20 Hettich at 5000×*g* for 15 min, and the plasma samples were separated and kept frozen at -20˚C in three Eppendorf tubes with distinct codes until the analysis was done. The participants returned on a nonconfined basis for continued pharmacokinetic blood sampling at 36, 48, and 72 h after drug administration in each period. After one-week washout, they were requested to return to the laboratory for the same blood sample analysis so as to complete the crossover design.

HPLC assay

The concentration of FA in plasma was determined using the HPLC method, developed and validated by Shah et al.[16] Corpus Fontium Historiae Byzantinae (CFHB) was used as the internal standard (IS). The method was verified before being used in the study. Stock solutions of 1 mg/mL were prepared for FA and CFHB and were diluted in methanol to obtain seven FA containing standard solutions of 0.05-20 µg/mL and one IS containing solution of 250 µg/mL. All of these solutions were then stored at -20˚C. The calibration curve was established by spiking the working standard solutions (50 µL) and the IS solution (50 µL) into drug-free human plasmas (450 µL). In relation to the concentration, matrix-matched FA solutions were prepared in plasmas at various concentrations of 0.05, 0.1, 0.5, 1, 5, 10, and 20 µg/mL, whereas IS solution was at concentration 250 µg/mL. A similar method was employed to prepare quality control (QC) samples in human plasmas. Four additional QC samples were of 0.05, 0.5, 10, and 15 µg/mL.

The analytical separation was performed on an Inertsil® C18 (4.6×150 mm, Waters) column, and the mobile phase was a gradient of acetonitrile and 0.01 M phosphate buffer pH 2.8 (75:25), with a flow rate of 1 mL/min that runs for 7 min. The samples were detected at 287 nm (Waters 2487 dual λ absorbance detector). The retention times for both FA and CFHB as the IS were 3.5 and 5.5 min, respectively. No interfering peaks were observed at either retention time. A typical chromatogram is shown in Figure 2. The limit of quantification for FA was 0.05 µg/mL. Plasma concentrations of FA were obtained from standard curves linear over a range of 0.05-20 µg/mL.

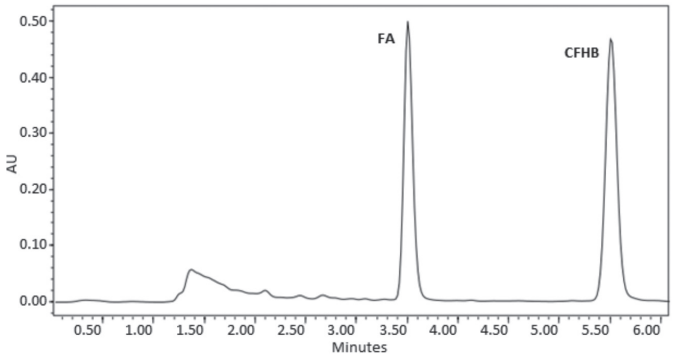

Figure 2. Chromatographic profiles of FA and CFHB as the internal standard in extracted human plasma

FA: Fenofibric acid, CFHB: 4'-chloro-5-fluoro-2-hydroxyl benzophenone

Plasma sample and preparation

Samples were prepared using the liquid-liquid extraction technique. Into 500 µL plasma sample were added 50 µL of IS solution (250 µg/mL) and 1 mL of 1 N HCl followed by mixing for 30 s in a vortex mixer. Then 3 mL of ethyl acetate was added and the mixture was mixed in a roller mixer for 30 min, followed by centrifugation for 15 min at 5000×*g*. The top organic layer was separated and evaporated for drying at 40˚C using a stream of nitrogen. The residue was reconstituted in 100 µL of the mobile phase and 60 µL was injected into the HPLC system (Waters 1525 binary pump).

Dissolution data analysis

DE was used for comparison of dissolution rates, calculated from the area under the dissolution curves at 60 min, and expressed as a percentage of the rectangle area described by 100% dissolution within the same time. Analysis of variance (ANOVA) was used to compare the DE of test and reference tablets profiles at 60 min (α=0.05).

Pharmacokinetic analysis

The pharmacokinetic parameters were calculated by a noncompartmental method. The elimination rate constant (K_{el}) was obtained from the least-square regression log linear portion (the last 3-5 points) of the plasma concentration/time profile. The area under the curve to the last measurable concentration (AUC_{0-t}) was calculated by the linear trapezoidal rule. The area under the curve extrapolated to infinity ($AUC_{0-\infty}$) was estimated with the equation $AUC_{0-t}+C_t/K_{el}$, where C_t is the last measured concentration. The peak plasma concentration (C_{max}) and the corresponding time to peak (T_{max}) were estimated by inspecting the individual drug plasma concentration/time profiles.

Statistical analysis

For the parameters of AUC_{0-t}, $AUC_{0-\infty}$, C_{max}, T_{max}, and $t_{1/2}$, ANOVA was applied for untransformed data. The level of significance was α=0.05 and a p value of <0.05 was considered statistically significant.

Correlation development

The principle of statistical moment analysis was utilized to assess the correlation of mean FA plasma concentration versus time in connection with ingestion of the three formulations. Mean dissolution time (MDT) was used to determine the correlation with *in vivo* mean residence time (MRT).

RESULTS

In vitro studies

All products fulfilled the general pharmaceutical requirements for weight variation, content assay, and content uniformity assay. The prepared tablets complied with the official specifications for disintegration time, hardness, and friability. The *in vitro* dissolutions were conducted in two different media (phosphate buffer pH 6.8 and biorelevant FaSSIF) and each at three different rotation speeds to determine their dissolution profile under various conditions.

The *in vitro* dissolution profiles of the SSD (F1) and conventional formulations (F2) are presented in Figure 3, and a summary of the mean DE_{60} of all FA tablets is given in Table 1. Significant differences existed between F1:F2 and F2:FA® in all conditions, whereas no significant difference arose from F1:FA® in 5 conditions.

In vivo studies

The concentration/time profiles of oral administration of both the SSD and conventional formulations and the reference formulation are depicted in Figure 4. All formulations resulted in an identical curve of plasma drug concentration versus time. The mean pharmacokinetic parameters of all FA tablets are summarized in Table 2. No significant difference was seen for any of the pharmacokinetic parameters from those formulations.

In vitro-in vivo relationship

Statistical moment analysis has been suggested as a better method to examine the IVIVC. A poor correlation between *in vivo* MRT and *in vitro* MDT for the three formulations was found in the present study (Figure 5).

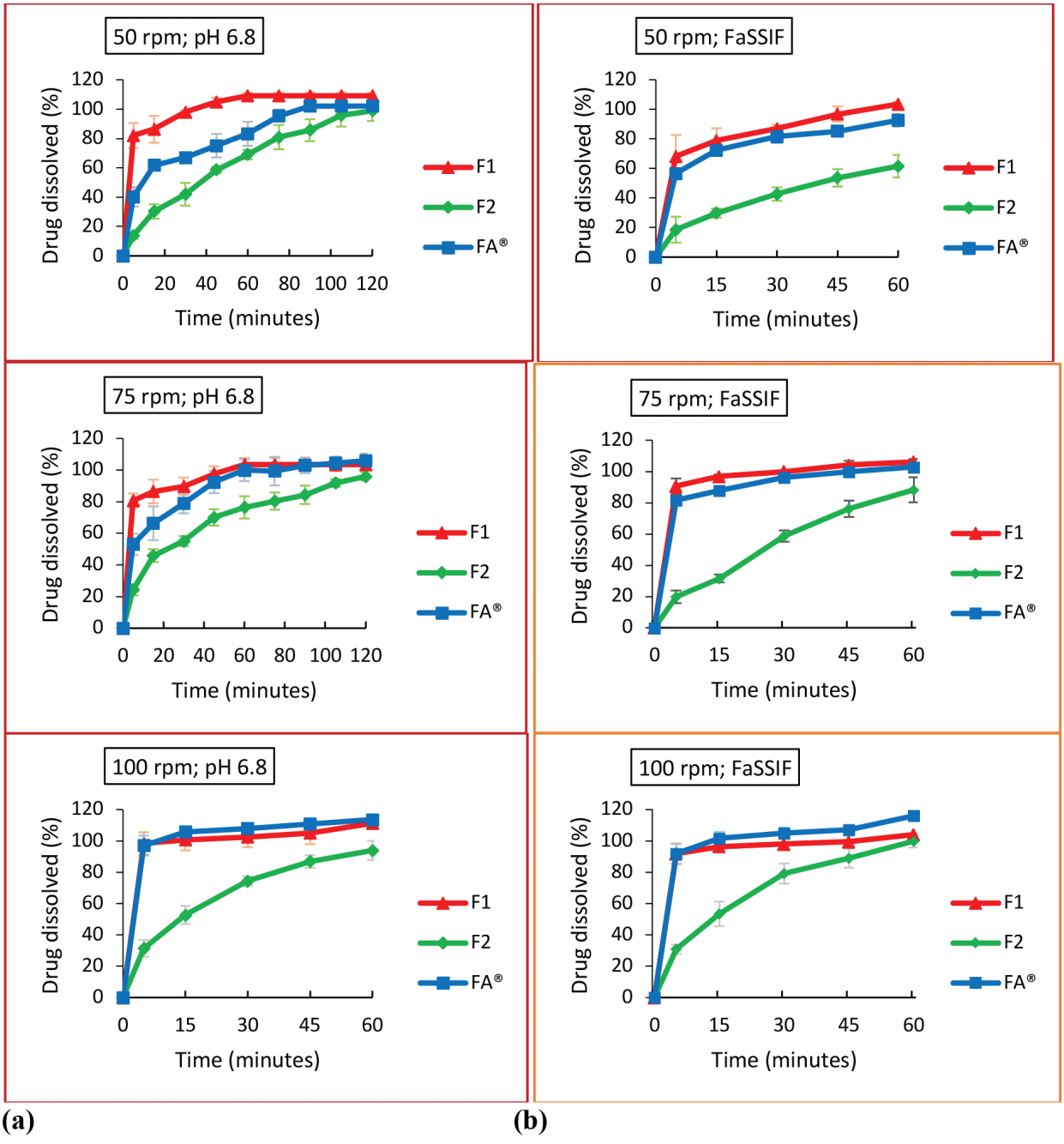

Figure 3. Dissolution profiles of SSD formulation (F1), conventional formulation (F2), and reference formulation (FA®) in phosphate buffer pH 6.8 (a) and FaSSIF biorelevant medium (b) at 50, 75, and 100 rpm

SSD: Surface solid dispersion, FA: Fenofibric acid, FaSSIF: Fasted state simulated intestinal fluid

DISCUSSION

The new formulation of FA, a BCS Class II drug, was developed in the present study and selected as an alternative to fenofibrate for oral administration. The SSD formulation was prepared by the solvent evaporation method to increase the dissolution of FA and compared to conventional and reference formulations. A conventional formulation of FA was prepared using wet granulation. All of these formulations met the general pharmaceutical requirements for physicochemical properties. However, significant differences were observed between them. The SSD formulation (F1) led to an instantaneous dissolution of the drug, releasing approximately 90% within the first 5 min in 75 and 100 rpm conditions. In contrast, the conventional formulation (F2) released nearly 80% of the drug within 45 min. Meanwhile, the reference formulation (FA®) yielded the same dissolution as F1. The FA dissolution from F1 increased due to the presence of the polymer and the physical structure of the SSD. In this case, FA was dispersed well on the CS surface, and the fine particles were able to increase its surface area for solubilization. When the CS came into contact with the dissolution medium, it caused swelling and made it possible for FA to be wet to dissolve in the media. The swelling of the CS caused cluster deaggregation of the drug particles and facilitated the dissolution process. Meanwhile, F2 could not achieve rapid dissolution despite the fact that around 80% of the drug dissolved within 45 min, and it satisfied the requirement for immediate drug release dosage form. Based on the data of the *in vitro* dissolution, there were significant differences found in the dissolution performances and therefore included in the development of the IVIVC.

The mean of all pharmacokinetic parameters from each product were not significantly different ($p>0.05$), suggesting that the plasma profiles generated by FA® were comparable to those produced by F1 and F2. The intrasubject CV was relatively small. Based on this analysis, F1 and F2 could be considered bioequivalent with FA®.

An appropriate condition of the dissolution study based on *in vivo* performance was adapted for routine and in process control for the FA formulation. The condition of dissolution in this study was similar to that proposed by the FDA (in pH 6.8 and 75 rpm) and correlated with the plasma profiles already obtained by performing bioavailability studies. Four correlation levels were defined in the IVIVC. It has been suggested to employ statistical moment analysis as a better method for examining the IVIVC. A level B correlation used all *in vitro* and *in vivo* data and it was therefore employed between MRT and MDT. There was no correlation (R^2=0.028) between MRT and MDT of the three formulations found in this study. Since the dissolution of the drug from F2 was slower than that of FA®, the IVIVC could not be achieved.

The *in vitro* dissolution behavior of FA did not reflect their *in vivo* properties in the fasted condition. The use of single medium dissolution for FA in the present study failed to create *in vivo* correlation. Moreover, a relatively significant difference was observed between the dissolution properties of both F1:F2 and F2:FA®. These formulations as *in vivo* bioequivalences are shown in Figure 4 and Table 2. Apparently, the dissolution media

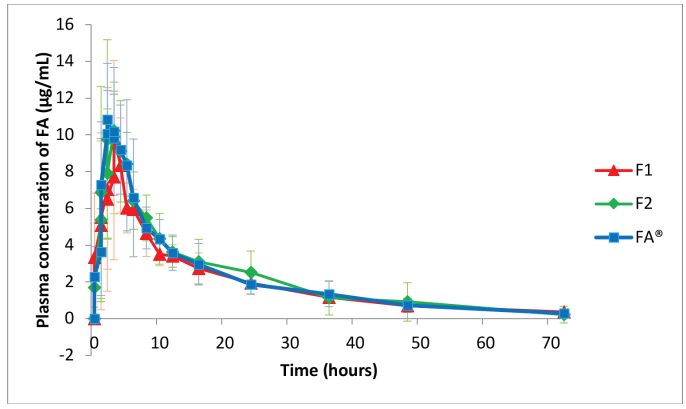

Figure 4. Average plasma concentration vs. time profiles of FA after oral administration (105 mg doses) of SSD formulation (F1), conventional formulation (F2), and reference formulation (FA®) in nine healthy male subjects. Data are shown as mean ± SD

FA: Fenofibric acid, SSD: Surface solid dispersion, SD: Standard deviation

Table 2. Pharmacokinetic parameters of fenofibric acid after single dose oral administration of three different formulations in nine healthy male subjects

Parameters	F1 (Mean ± SD)	F2 (Mean ± SD)	FA® (Mean ± SD)
AUC_{0-72} (µg.h/mL)	136.94±30.85	157.57±55.81	150.57±40.49
$AUC_{0-\infty}$ (µg.h/mL)	148.45±34.62	171.09±62.95	158.22±42.14
C_{max} (µg/mL)	11.79±3.72	12.94±3.95	14.12±2.68
T_{max} (h)	2.99±0.39	2.67±0.41	2.63±0.35
$T_{1/2}$ (h)	20.97±3.36	19.72±6.65	17.25±4.12

SD: standard deviation, FA: Fenofibric acid

Table 1. Dissolution parameters (DE_{60}) of fenofibric acid from three formulations at six conditions

Code	DE_{60} (%) ± SD					
	50 rpm		75 rpm		100 rpm	
	pH 6.8	FaSSIF	pH 6.8	FaSSIF	pH 6.8	FaSSIF
F1	92.55±3.50	85.05±1.53	87.88±4.96	95.78±1.79	99.11±6.07	93.89±3.26
F2	41.55±1.64	40.29±4.31	53.44±3.89	53.77±2.57	66.97±3.29	69.50±4.38
FA®	63.79±3.71	75.35±1.34	75.81±5.91	90.43±1.21	93.11±0.73	96.23±3.03

DE: Dissolution efficiency, SD: Standard deviation, FaSSIF: Fasted state simulated intestinal fluid, FA: Fenofibric acid

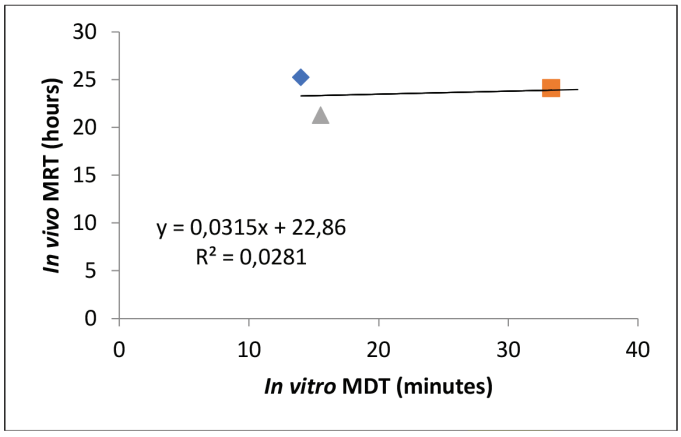

Figure 5. Correlation between MDT of in vitro dissolution (in pH 6.8; 75 rpm) and MRT of plasma drug concentration from three formulations F1 (◆), F2 (■), and FA® (▲)

MDT: Mean dissolution time, MRT: Mean residence time, FA: Fenofibric acid

in this study did not completely simulate the conditions of the GI tract. It is reported that a biorelevant dissolution medium has the ability to predict well the *in vivo* performances of insoluble drugs. However, that purpose was not achieved in the present study. Further studies are suggested using biorelevant pH gradient methods to obtain a strong IVIVC.

In most cases, statistically significant differences of *in vivo* MRT among various formulations were not significant enough to produce a strong correlation between MRT and MDT. For a 105 mg dose of FA and aqueous solubility of 0.162 mg/mL, 650 mL of fluid was required to dissolve a single dose. Therefore, the volume of water taken initially not only dissolved the drug to a great extent but also decreased the dependency of drug absorption on drug dissolution.[17] This phenomenon led to a nil correlation in the present study. The fact that the *in vitro* differences in the early dissolution were not realized in the *in vivo* differences was attributed to the continuous excretion of bile that happened in the GI tract.[18] There was still a possibility that FA was absorbed with the help of a transporter (facilitated transport) and/or energy (active transport). However, the amount was likely to be limited, even if much was dissolved.

Study limitation

The present study was limited by its use of a single medium method for dissolution testing. Further studies are suggested to use biorelevant pH gradient methods to obtain a strong IVIVC.

CONCLUSION

The *in vitro* dissolution behavior of FA using a single medium did not reflect its *in vivo* properties in the fasted condition. There was no correlation between the *in vitro* dissolution and the *in vivo* bioavailability of FA in this condition.

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REFERENCES

1. Alagona P. Fenofibric acid: a new fibrate approved for use in combination with statin for the treatment of mixed dyslipidemia. Vasc Health Risk Manag. 2010;6:351-362.
2. Keating GM, Croom KF. Fenofibrate: a review of its use in primary dyslipidaemia, the metabolic syndrome and type 2 diabetes mellitus. Drugs. 2007;67:121-153.
3. Ling H, Luoma JT, Hilleman D. A review of currently available fenofibrate and fenofibric acid formulations. Rev Cardiol Res. 2013;4:47-55.
4. Rath NP, Haq W, Balendiran GK. Fenofibric acid. Acta Crystallogr Sect C Cryst Struct Commun. 2005;61:81-84.
5. Godfrey AR, DiGiacinto J, Davis MW. Single-dose bioequivalence of 105-mg fenofibric acid tablets versus 145-mg fenofibrate tablets under fasting and fed conditions: a report of two phase I, open-label, single-dose, randomized, crossover clinical trials. Clin Ther. 2011;33:766-775.
6. Zhu T, Ansquer JC, Kelly MT, Sleep DJ, Pradhan RS. Comparison of the gastrointestinal absorption and bioavailability of fenofibrate and fenofibric acid in humans. J Clin Pharmacol. 2010;50:914-921.
7. Wei X, Li P, Liu M, Du Y, Wang M, Zhang J, Wang J, Liu H, Liu X. Absolute oral bioavailability of fenofibric acid and choline fenofibrate in rats determined by ultra-performance liquid chromatography tandem mass spectrometry. Biomed Chromatogr. 2017;31.
8. Rautio J. Prodrugs and Targeted Delivery: Towards Better ADME Properties. Weinheim, Germany; Wiley-VCH Verlag GmbH & Co; 2011:103-104.
9. Kim KS, Kim JH, Jin SG, Kim DW, Kim DS, Kim JO. Effect of magnesium carbonate on the solubility, dissolution and oral bioavailability of fenofibric acid powder as an alkalising solubilizer. Arch Pharm Res. 2016;39:531-538.
10. Tsume Y, Mudie DM, Langguth P, Amidon GE, Amidon GL. The Biopharmaceutics Classification System: Subclasses for *in vivo* predictive dissolution (IPD) methodology and IVIVC. Eur J Pharm Sci. 2014;57:152-163.
11. Salem HF, Elbary AA, Maher ME. *In vitro* and *in vivo* evaluation of glibenclamide using surface solid dispersion (SSD) approach. Br J Pharmacol Toxicol. 2011;2:51-62.
12. Khatry S, Sood N, Arora S. Surface solid dispersion - a review. Int J Pharm Sci Nanotechnol. 2013;6:1915-1924.

13. Windriyati YN, Sumirtapura YC, Pamudji JS. Dissolution enhancement and physicochemical characterization of fenofibric acid in surface solid dispersion with croscarmellose sodium. J Res Pharm. 2019;23:315-325.

14. Kim KS, Jin SG, Mustapha O, Yousaf AM, Kim DW, Kim YH, Kim JO. Novel fenofibric acid-loaded controlled release pellet bioequivalent to choline fenofibrate-loaded commercial product in beagle dogs. Int J Pharm. 2015;490:273-280.

15. Niraimathi V, Suresh AJ, Alageswaran A. UV spectrophotometric determination of fenofibric acid by using hydrotropy. Int J Pharma Sci Res. 2015;6:451-458.

16. Shah I, Barker J, Barton SJ, Naughton DP. A novel method for determination of fenofibric acid in human plasma using HPLC-UV: application to a pharmacokinetic study of new formulations. J Anal Bioanal Tech. 2014;S12:10-13.

17. Emami J. Comparative *in vitro* and *in vivo* evaluation of three tablet formulations of amiodarone in healthy subjects. Daru J Pharm Sci. 2010;18:193-199.

18. Do TT, Van Speybroeck M, Mols R, Annaert P, Martens J, Van Humbeeck J, Vermant J, Augustijns P, Van den Mooter G. The conflict between *in vitro* release studies in human biorelevant media and the *in vivo* exposure in rats of the lipophilic compound fenofibrate. Int J Pharm. 2011;414:118-124.