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Design of fenofibrate microemulsion for improved bioavailability

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ABSTRACT

The objective of the present study was to formulate a microemulsion system for oral administration to improve the solubility and bioavailability of fenofibrate. Various formulations were prepared using different ratios of oils, surfactants and co-surfactants (S&CoS). Pseudo-ternary phase diagrams were constructed to evaluate the microemulsification existence area. The formulations were characterized by solubility of the drug in the vehicles, mean droplet size, and drug content. The stability was also investigated by store for 3 months under 4 °C, 25 °C and 40 °C and diluted 100 times for 3 days. The optimal formulation consists of 25% Capryol 90, 27.75% Cremophore EL, 9.25% Transcutol P and 38% water (w/w), with a maximum solubility of fenofibrate up to ~40.96 mg/mL. The microemulsion was physicochemical stable and mean droplet size was about 32.5–41.7 nm. The pharmacokinetic study was performed in dogs and compared with Lipanthy® capsule. The result showed that microemulsion has significantly increased the C_{max} and AUC compared to that of Lipanthy® capsule ($p < 0.05$). The oral bioavailability of fenofibrate microemulsions (FEN-MEs) in ME-3 and ME-4 were 1.63 and 1.30-fold higher than that of the capsule. Our results indicated that the microemulsions could be used as an effective formulation for enhancing the oral bioavailability of fenofibrate.

1. Introduction

Fenofibrate, a poorly water-soluble drug, is widely used as one of the best lipid-lowering drugs. Fenofibrate has a wide range of effects on the synthetic and catabolic pathways of cholesterol and triglyceride metabolism (Yun et al., 2006). It is a neutral lipophilic compound ($\log P = 5.24$) with a very low aqueous solubility (<0.5 mg/L) (Munoz et al., 1994). Fenofibrate is a prodrug that is converted rapidly after oral administration through the hydrolysis of the ester bond to fenofibric acid, the active form and major metabolite of fenofibrate (Fig. 1) (Najib, 2002). Its main drawback has been the low bioavailability of the active metabolite, fenofibric acid, when the prodrug is taken orally on an empty stomach (Kearing and Ormrod, 2002; Adkins and Faulds, 1997; Guay, 2002). Recently, some studies reported that the bioavailability of poorly soluble fenofibrate can be improved by employing different drug delivery systems. Laboratoires Fournier SA (Dijon, France) has developed a micronized fenofibrate capsule and obtained a relatively high bioavailability. Self-microemulsifying drug delivery system containing fenofibrate could also obtain a better pharmacodynamic potential (Patel and Vavia, 2007). Furthermore, fenofibrate solid dispersion prepared by hot-melt extrusion and immediate-release tablets involving wet grinding were novel formulations to improve the dissolution and bioavailability (He et al., 2010; Zhang et al., 2010).

Among the various drug delivery systems, microemulsion may be a better choice to solve these problems. Microemulsion is defined as a monodispersion spherical droplets consisting of oil, surfactant, co-surfactant and aqueous phase, which is optically isotropic and thermodynamically stable with a droplet diameter within the range of 10–100 nm (Tenjarla, 1999). Microemulsions could enhance the potential solubilization of lipophilic drugs. Employing microemulsion formulation improved oral bioavailability of tonitrendipine as indicated in its AUC values which were more than three times larger compared to those of its oil solution (Kawakami et al., 2002). A new microemulsion formulation resulted in a 5.2-fold higher oral bioavailability of docetaxel in rats compared to that of oral Taxotere® (Yin et al., 2009). Presenting the drug in the dissolved form using lipid-based formulations provides significant improvement of oral absorption as compared to an oral solid or suspension dosage form (Narang et al., 2007).

The main objective of this study is to formulate an o/w microemulsion system of fenofibrate for oral administration. According to a solubility study and pseudo ternary phase diagrams, the formulation composed of various vehicles in different ratios were investigated. And droplet size, stability after dilution were performed for the optimized formulation. In addition, different

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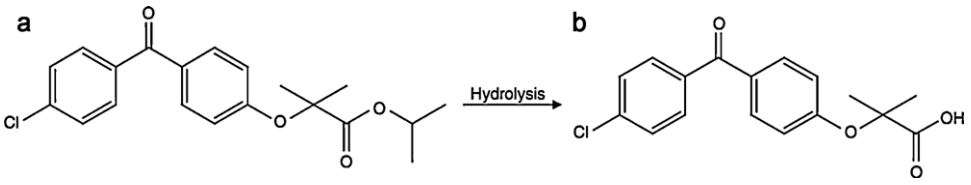

Fig. 1. Molecular structure of (a) fenofibrate and (b) its active metabolite fenofibric acid.

formulations were compared by the evaluation of the pharmacokinetics.

2. Materials and methods

2.1. Materials

Fenofibrate was purchased from Kaifeng Pharmaceutical Co. Ltd. (Henan, China). While Lipanthyl® capsule was purchased from Laboratoires Fournier SA. Maisine 35-1, Plurol OleiqueCC 497, Capryol 90, Labrafil M 1944 CS were received as gifts from Gattefossé Co. (Shanghai, China), while Labrasol, Transcutol P was purchased from the same company. Cremophor RH and Cremophor EL were purchased from Xietai Chemical Co. Ltd. (Shanghai, China). Tween 80 and PEG 400 were obtained from Huadong Chemical Co. (Tianjin, China). Methanol was HPLC grade and supplied from Kermel Chemical Co. Ltd. (Tianjin, China). Double-distilled water was used throughout the study. All other chemicals are HPLC or analytical grade.

2.2. Preparation of microemulsion

2.2.1. Solubility study

The solubility of fenofibrate in various vehicles was determined by adding excess amount of fenofibrate into 1 mL of each vehicle in a centrifugal tube, followed by mixing in a shaking incubator at 25 °C for 3 days. The samples were centrifuged to remove the excess drug. The fenofibrate in the supernatant was diluted with methanol and measured by HPLC mentioned below after filtrated by a 0.45 μm filter.

2.2.2. Construction of pseudo-ternary diagrams

In order to find out the ratio of components for the area of microemulsion existence, pseudo-ternary phase diagrams were constructed using water titration method at 25 °C. Surfactant and co-surfactant were blended into each tube at specific weight ratios as 1:1, 2:1 and 3:1, then were vortexed vigorously for 1 min to make the surfactant mixture. The ratios of oil phase to the mixture of surfactant and co-surfactant were changed from 9:1 to 1:9 (w/w). Distilled water was added drop by drop to the mixture of oil, surfactant and co-surfactant under gentle magnetic stirring. The appearances from clear to turbid and turbid to clear were investigated, respectively. Based on these diagrams, appropriate oils, S&CoS were selected for the preparation of fenofibrate microemulsions (FEN-MEs).

2.2.3. Preparation of FEN-MEs

FEN-MEs were prepared at desired component ratios. Excess fenofibrate was added to the mixtures of oil, surfactant and co-surfactant with varying ratios mentioned before. Then water was added to the mixture dropwise and stirring for 24 h at 25 °C. The undissolved drug was removed by centrifugation and the supernatant was filtered by 0.45 μm membrane. The concentration in the filtrate was measured by HPLC.

2.2.4. HPLC analysis of fenofibrate

The HPLC analysis system consisted of a LC-20AT pump and SPD-20A UV/VIS detector (Shimadzu, Kyoto, Japan) and the chromatographic column was a Kromasil C-18 (5 μm, 250 mm × 4.6 mm). Mixture of acetonitrile:water (70:30, v/v) was used as the mobile phase and adjusted to pH 2.5 with phosphoric acid. The flow rate was 1.0 mL/min; UV-detection was at a wavelength of 287 nm.

2.3. Characterization of microemulsions

2.3.1. Droplet size

The average size and distribution of FEN-MEs were determined at 25 °C by photon correlation spectroscopy (PCS) using a NICOMP particle sizing system (CW380, Santa Barbara, CA) at a fixed angle of 90°. The analysis data of droplet size were evaluated using the volume distribution.

2.3.2. Morphology detection by TEM

The morphology of FEN-MEs was investigated by transmission electron microscopy (TEM) (JEM-100SX, JEOL, Tokyo, Japan). One drop of diluted samples was negatively stained by 2% phosphotungstic acid (PTA) and placed on film-coated copper grids followed by drying at 25 °C before examination under the TEM.

2.3.3. Stability of microemulsions

The FEN-MEs were stored at 4 °C, 25 °C and 40 °C for 3 months. Furthermore, each formulation of FEN-MEs was diluted 100 times with distilled water for 3 days. The stability was investigated by observing the occurrence of the dispersed phase or crystal after centrifugation.

2.4. Pharmacokinetics studies

Prior to the oral administration, the microemulsions were filled into 0 size hard gelatin capsules. Six healthy male dogs were randomized to be administered 200 mg orally with marked capsule Lipanthyl® capsule and microemulsions, respectively. After administration, the blood samples were collected in tubes containing heparin at 0, 0.5, 1, 2, 3, 4, 5, 6, 8, 10, 12, 18, 24, 48 and 72 h. Samples were centrifuged for 10 min after collection and stored at −20 °C prior to analysis.

Measurement of fenofibric acid was done by liquid–liquid extraction using included the addition of 50 μL internal standard to 1 mL plasma sample with 5 mL ethyl ether and adjusted to pH 2.5 with phosphoric acid. After centrifuging for 5 min, the supernatant was dried at 40 °C under a stream of nitrogen gas. The residue was reconstituted into 100 μL methanol and 20 μL was injected into the HPLC column and determined by the method mentioned above.

2.5. Statistical analysis

Pharmacokinetic parameters were analyzed using established non-compartmental methods. C_{max} and T_{max} were obtained directly from these curves. AUC_{0-t} was calculated using the trapezoidal method. $AUC_{0-\infty}$ was calculated by: $AUC_{0-\infty} = AUC_{0-t} + C_t/K_e$, where C_t is the plasma concentration observed at 72 h and K_e was the apparent elimination rate constant obtained from the terminal slope of the individual plasma concentration–time curves after

logarithmic transformation of the plasma concentration values and application of linear regression, the biological half life ($T_{1/2}$) was calculated by: $T_{1/2} = 0.693/K_e$.

The statistical differences between the two formulations were assessed using a Student's *t*-test. Mean ± SD and the statistically data were considered statistically significant at $p < 0.05$.

3. Results and discussion

3.1. Solubility study

The solubility of fenofibrate in various oils, S&CoS is tabulated in Table 1. Drug may be solubilized in the oily core and/or on the

Table 1
Solubility of fenofibrate in various vehicles at 25 °C (mean ± SD, $n = 3$).

	Vehicle	Solubility of fenofibrate (mg/mL)
Oil	Capryol 90	154.48 ± 4.24
	Plurol OleiqueCC 497	18.26 ± 2.24
	Labrafil M 1944 CS	102.91 ± 3.91
	MCT	79.26 ± 2.95
Surfactant	Tween 80	102.81 ± 3.81
	Cremophore EL	91.7 ± 2.29
	Span 20	47.02 ± 1.69
	Cremophor RH	54.37 ± 1.85
Co-surfactant	Transcutol P	204.36 ± 6.70
	PEG 400	74.11 ± 1.96
	Ethanol	43.03 ± 1.31

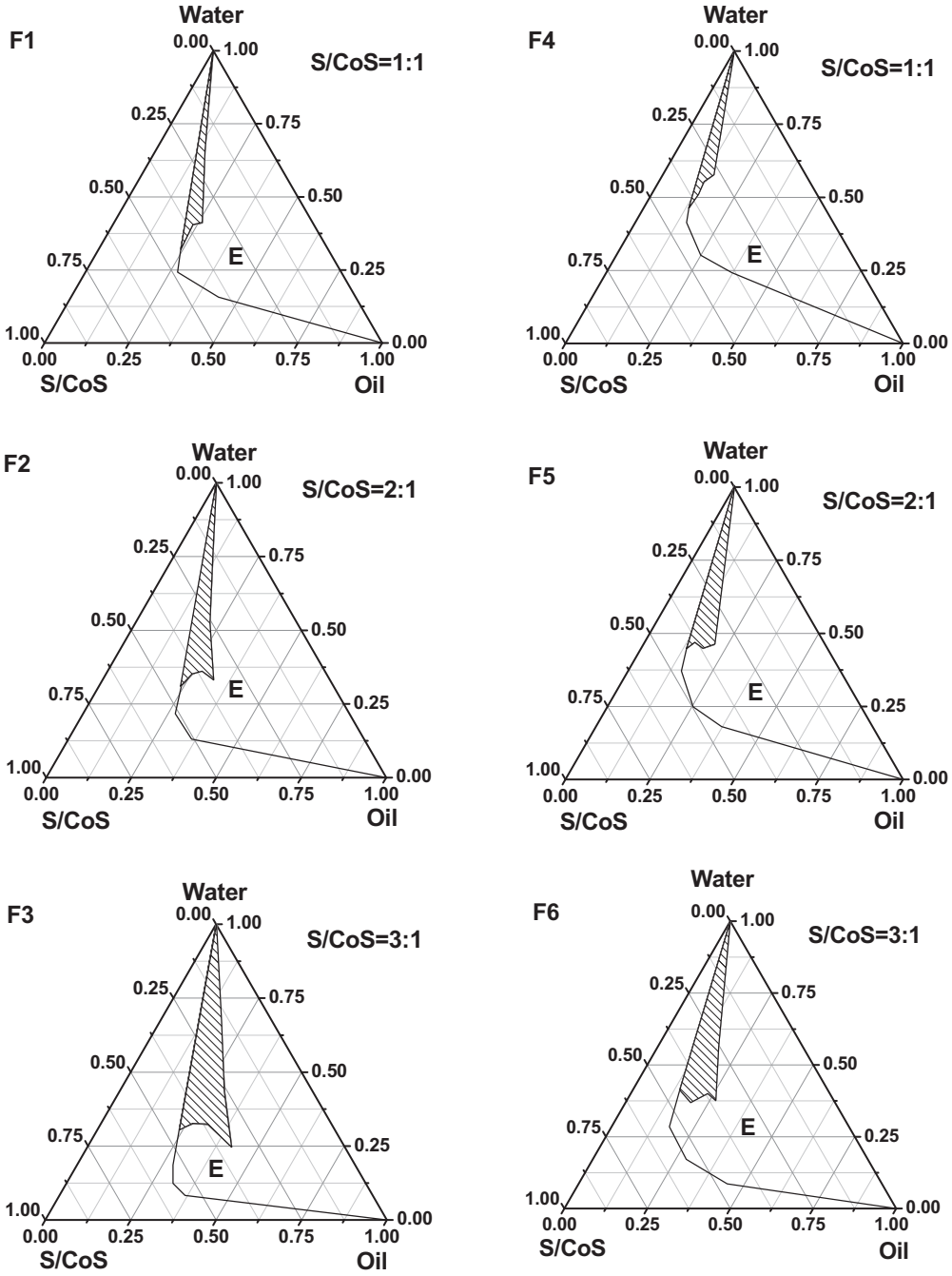

Fig. 2. Pseudo-ternary phase diagrams consisted of following components: oil = Capryol 90 (F1, F2 and F3) or Labrafil M 1944 CS (F4, F5 and F6), S&CoS = Cremophore EL and Transcutol P. Shaded region = microemulsion; S/CoS indicates the ratio of S&CoS; E=emulsion.

interface of these structures, so the vehicles which were selected should have a better solubility to the drug. Based on the results, Capryol 90 and Labrafil M 1944 CS showed the highest solubility in these oils. Among these S&CoS, Tween 80 and Transcutol P showed better solubility for fenofibrate than others. Cremophor RH was semi-solid at room temperature, so the solubility in it should be heated at 40 °C before determined. Furthermore, Cremophore EL, Cremophor RH and PEG 400 were still were chosen for ternary phase diagram study. All these vehicles were commercial available and low toxicity.

3.2. Pseudo ternary phase diagram study

The construction of phase diagram makes it easy to find out the maximum proportion of oil for the existence area of microemulsions (Piao et al., 2010). Although Tween 80 has better solubility among surfactants, microemulsions is hard to formulate when Capryol 90 or Labrafil M 1944 CS was selected as oil whatever co-surfactant was used. The formulation was not transparent and stable when Labrasol used as co-surfactant with Cremophor EL after diluted with distilled water. While the systems containing Cremophor EL as surfactant and Transcutol P as co-surfactants formed a stable and broad microemulsion area. So Transcutol P was selected as co-surfactants for its good emulsifying ability and high solubility.

So in this study, Capryol 90 and Labrafil M 1944 CS was selected as oil phase, Cremophor EL and Transcutol P as S&CoS. A total of six phase diagrams were constructed at different ratios of S&CoS (1:1, 2:1 and 3:1). The results of phase diagram were presented in Fig. 2. Phase behavior investigation of F1, F2 and F3 revealed that the area of microemulsion enlarged as the ratio of surfactant increased. Compared the formulations (Labrafil M 1944 CS as oil phase) at the same ratio of S&CoS, we found that the formulation composed by Capryol 90, Cremophor EL and Transcutol P have a broader microemulsion area. Furthermore, the solubility of fenofibrate in Capryol 90 was significantly higher than that in Labrafil M 1944 CS. It may affect the drug content of microemulsion. For these researches of various components and ratio of S&CoS, Cremophor EL and Transcutol P was determined as S&CoS at the ratios of 3:1. Based on the results, four microemulsions with the different ratios of oil:Sm:water (w/w/w) were selected for further experiments: (i) Capryol 90 as oil phase: ME-1 (17:37:36), ME-2 (19:38:43) ME-3 (25:37:38); (ii) Labrafil M 1944 CS as oil phase: ME-4 (26:39:35) (see Table 2).

3.3. Characterization of microemulsions

Characterization of microemulsions of three formulations at following oil:S&CoS:water (w/w/w) ratio were listed in Table 2. Morphology of ME-3 was characterized using TEM (Fig. 3).

Microemulsions were transparent with sky-blue opalescent. As shown in Table 2, the drug content and mean droplet size were mainly attributing to the ratio of a significant amount of oil. The ME-3 produce the highest solubility of fenofibrate (40.96 mg/mL) compared to that in aqueous solution (<0.5 mg/L), and the mean droplet size was ~41.7 nm.

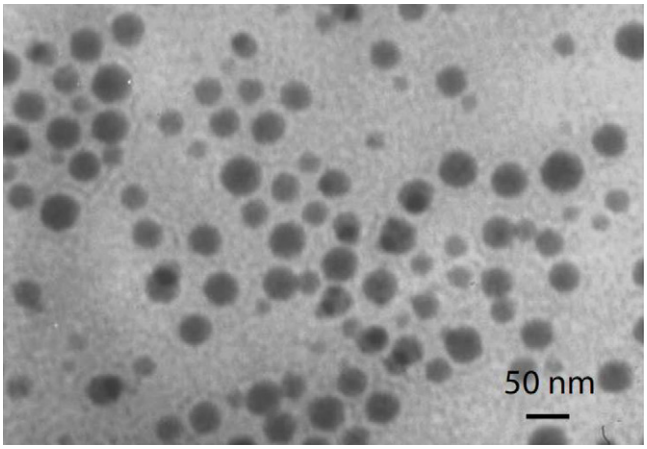

Fig. 3. Transmission electron microphotography of ME-3.

Table 3
Pharmacokinetic parameters among ME-3, ME-4 and Lipanthyl® capsule (mean ± SD, $n = 6$).

Group	Lipanthy® capsule	ME-3	ME-4
$T_{1/2}$ (h)	23.18 ± 4.13	20.53 ± 3.77	20.83 ± 2.07
C_{max} (mg/L)	24.46 ± 2.93	70.20 ± 8.26	49.05 ± 6.71
t_{max} (h)	2.67 ± 0.82	2.33 ± 0.52	2.83 ± 0.41
AUC (h mg/L)	490.38 ± 44.01	800.52 ± 125.44	639.44 ± 86.95
MRT (h)	29.08 ± 3.08	20.94 ± 1.09	26.37 ± 2.43

During this study, fenofibrate was added to the mixture of oil, S&CoS and then microemulsified to find an optimized formulation with higher drug-loading capacity. After microemulsified, drug may be solubilized in the oily core and/or on the interface of these structures. The phenomenon of drug solubilization at the interface affects not only drug loading capacity but also drug precipitation upon dilution (Narang et al., 2007). To avoid precipitation and crystallization, the microemulsions was store at 4 °C, 25 °C and 40 °C for 3 months and diluted 100 times for 3 days to examine its stability. All the three formulations have no precipitation and crystallization after the operation above. This indicates that FEN-MEs were physicochemical stable. The improvement of drug content using o/w microemulsion depends on the solubility of the drug in the dispersed oil phase and the percentage of that phase present (Malcolmson and Lawrence, 1993). Hence, the solubility of ME-3 produced a higher solubilizing capacity of fenofibrate than ME-1, ME-2 and ME-4. Finally, the solubility of fenofibrate, a poorly water soluble drug, was promoted to 40.96 mg/mL by optimum microemulsion formulation consisted of Capryol 90 25%, Cremophor EL 27.75%, Transcutol P 9.25% and water 38% (w/w).

3.3.1. Pharmacokinetic study

Fig. 4 shows mean plasma concentration–time curve of fenofibric acid after a single oral administration of three formulations. The oral pharmacokinetic parameters are presented in Table 3. Obviously, after oral administration, ME-3 and ME-4 exhibited the higher absorption, with greater C_{max} (70.20 mg/L and 49.05 mg/L)

Table 2
Compositions, droplet size, and fenofibrate content of the selected formulations (mean ± SD, $n = 3$).

Formulation	Oil	S&CoS	Oil:S&CoS:water (%) (S/CoS = 3:1)	Mean droplet size (nm)	Drug content (mg/mL)
ME-1			17:47:36	32.5 ± 2.4	25.65 ± 0.22
ME-2	Capryol 90	Cremophore EL & Transcutol P	19:38:43	35.6 ± 2.5	31.92 ± 0.20
ME-3			25:37:38	41.7 ± 3.2	40.96 ± 0.32
ME-4	Labrafil M 1944 CS	Cremophore EL & Transcutol P	26:39:35	40.3 ± 2.9	35.26 ± 0.29

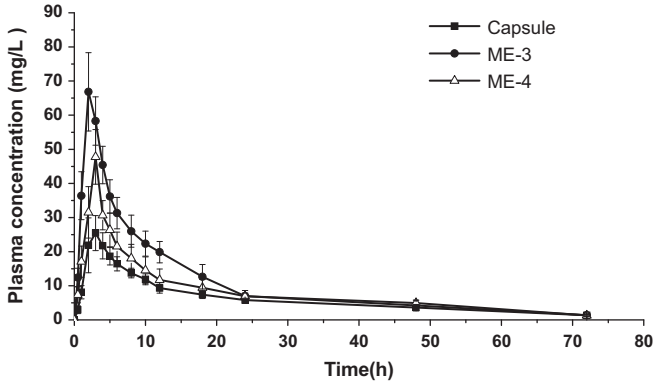

Fig. 4. The blood concentration–time profile of fenofibric acid after oral administration of ME-3, ME-4 and Lipanthyl® capsule to dogs (mean ± SD, $n = 6$).

and AUC_{0-t} values (800.52 h mg/L and 639.44 h mg/L), respectively, than that of Lipanthyl® capsules. The bioavailability of ME-3 and ME-4 was approximately 1.63 and 1.30-fold higher than that of capsule.

The significant differences of the factors leading drug absorption in vivo between the microemulsion preparations and Lipanthy® capsule were probably attributed to the following: fenofibrate is often assumed to be a BCS Class II drug, the oral absorption of fenofibrate is mainly limited by the dissolution rate of the formulation. Reduction in the particles size is a key factor for improving the oral absorption of these drugs. In microemulsion formulations, the particle size range was reduced to about 40 nm, resulting in an increase in surface area and saturation solubility. However, Lipanthyl® capsule, micronized fenofibrate could not dissolute rapidly in the gastrointestinal tract, the decrease of the delivery of fenofibrate can achieve low drug concentrations in the gastrointestinal tract thus poor oral absorption.

It has also been reported that in the fasted state, absorption of fenofibrate from the microsized formulation the dissolution of fenofibrate appears to be rate-determining, while for the nanosized formulation is at least partly permeability-limited (Juenemann et al., 2011). So there are other reasons determining fenofibrate oral absorption. It is reported that absorption of fenofibrate is increased by ~35% when it is administered with high fat food rather than in a fasting state (Tricor, 2002). As we all known, the constituents of microemulsion includes oil, surfactant and co-surfactant, so the addition of oil can be viewed as an option for improving oral bioavailability of fenofibrate. Compared with the ME-3 and ME-4, consist of different oil, Capryol 90 showed higher absorption than that of Labrafil M 1944 CS.

The surfactant and co-surfactant (Cremophor EL and Transcutol P) may have contributed to an increase in the permeability of the intestinal membrane, or improved the affinity between lipid particles and the intestinal membrane. Further, due to small particle size, FEN-MEs may adhere to the gut membrane or enter the intervillar spaces thus extending gastrointestinal residence time in the gastrointestinal tract.

Although the microemulsion showed enhanced bioavailability of fenofibrate, because drug content was about or below 40 mg/mL, seven or eight capsules were used to the dogs, so how to increase the drug content in the microemulsion, formulated it as a preparation for oral use, and further studies of this field are needed.

4. Conclusion

The microemulsion formulation is physically stable by storage for 3 months under 4 °C, 25 °C and 40 °C and diluted 100 times for 3 days. The oral bioavailability of ME-3 and ME-4 was approximately 1.63 and 1.30-fold higher compared to that of the Lipanthy® capsule. This result was mainly due to the higher solubility and bioavailability of microemulsion system. The optimum ME-3 consisted of Capryol 90 25%, Cremophore EL 27.75%, Transcutol P 9.25% and water 38% (w/w) exhibited the highest solubility of fenofibrate (40.96 mg/mL). In conclusion, the new employment of microemulsion for oral administration of fenofibrate can be used as a suitable carrier system. Furthermore, toxicological study of microemulsion should be further investigated.

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