Effects of Ingested Water Volume on Oral Absorption of Fenoibrate, a BCS Class II Drug, from Micronized and Amorphous Solid Dispersion Formulations in Rats

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In this study, we investigated the effects of ingested water volume on the oral absorption of fenoibrate (FEN) with several formulations to confirm the applicability of rats for oral formulation screening. Oral absorption of suspended crystalline FEN was significantly improved by increasing ingested water volume (from 0.5 to 2 mL). FEN absorption improvement by particle size reduction and the linearity in oral absorption by dose escalation suggested that the rate-limiting step of FEN absorption in rats was the dissolution rate, consistent with that in humans. When FEN, as an amorphous solid dispersion (ASD) formulation, was suspended in water followed by immediate administration, oral FEN absorption was significantly higher than when administered in crystalline form and was not influenced by the differences in ingested water volume. Oral absorption of FEN from encapsulated ASD formulation in 1 or 2 mL of water was comparable with that of the suspension form. However, 0.5 mL of water significantly reduced the oral absorption of the solid ASD FEN formulation. These results indicate that to improve the oral absorption of poorly water-soluble drugs when performing a preclinical study with rats, 1 mL of water is the minimum preferable ingested volume to evaluate in vivo formulation performance.

Key words amorphous solid dispersion; fenoibrate; ingested water volume; micronized formulation; oral absorption; poorly water-soluble drug

INTRODUCTION

Oral absorption of drugs with poor water-solubility is strongly affected by gastrointestinal (GI) conditions, such as gastric acidity, food state, and bile concentration.1–5 Therefore, to fully understand the effects of these conditions in humans on oral drug absorption, in vitro and in vivo studies must be explored. The biopharmaceutics classification system (BCS) is useful when considering factors that affect drug absorbability.6 An important quantity in these evaluations is dose number. Dose number is the mass of the drug divided by the product of the administered water volume and solubility. This value is used to determine the threshold separating high or low solubility drugs. Both Steingöetter et al. and Mudie et al. used magnetic resonance imaging to investigate gastric emptying after ingestion of 300 mL of water and the distribution of GI fluid volumes following a 240 mL dose of water, respectively.7,8 When subjects, after fasting, drank 240 or 300 mL of water, the maximum fluid volume in the stomach was evaluated to be 242 ± 9 mL8 or 296 mL (range of 279–323).7 After water intake, the gastric fluid was observed to rapidly move to the small intestine. These findings suggest that the ingested water volume directly influences the dispersion of oral formulations and the dissolution of drugs in the GI tract. However, the effect of water volume on drug absorption is still unclear and requires further assessment to develop effective formulations, especially for drugs with poor water solubility.

In preclinical studies, it is essential to accurately evaluate the oral absorption of drugs with poor water-solubility, like BCS class II drugs, in conjunction with formulation effects to facilitate successful oral drug development. The effect of ingested water volume on oral drug absorption is not sufficiently discussed in preclinical studies. In a previous study, we examined the effect of ingested water volume for immediate-release formulations, capsules and mini-tablets, for the oral absorption of water-soluble drugs, classified as BCS classes I and III, in rats.9 The ingested water volume markedly influenced the rate of oral absorption of drugs administered in solid forms but not in solution. We concluded that 1 mL of water was optimal for the co-administration of solid drug forms to evaluate the oral absorption of high solubility drugs. However, the impact of ingested water volume on the oral absorption of poorly water-soluble drugs was not explored, in addition to evaluating formulations for improved oral absorption.

In this study, rats were orally administered a BCS class II drug in varying volumes of water to clarify how the ingested water volume influences the oral absorption of BCS class II drugs in different forms. Fenoibrate (FEN), a BCS class II drug,10 was selected as a model drug and, due to its poor water solubility and neutral characteristics, is frequently used to demonstrate formulation technologies.11–14 The plasma concentration of fenofibrin acid (FA), an active metabolite of FEN, was measured to evaluate oral FEN absorption. Currently, several formulations, such as micronized, nanosized, and amorphous solid dispersions (ASD), have been developed for clinical formulations.15–19 Increasing the dissolution rate of FEN by such formulation technologies improves oral availability and enables dose size reductions for clinical treatment. It is important to evaluate these effects on oral absorption accurately in preclinical studies. Therefore, the effects of micronized and ASD formulations on oral FEN absorption were
examined in rats, and the effect of the ingested water volume was also evaluated.

MATERIALS AND METHODS

Materials  FEN and FA were purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). Lactose and 0.5% (w/v) methylcellulose solution were obtained from FUJIFILM Wako Pure Chemical Corporation (Osaka, Japan). Tricor® Tablets (80mg) were purchased from TEIJIN PHARMA LIMITED (Tokyo, Japan). Size 9 gelatin capsules (PCCaps®) were supplied from Capsugel® Japan Inc. (Kanagawa, Japan).

Preparation of Formulations  Two FEN crystalline forms with a narrow particle size of 300–500 µm (mean particle size: 400 µm) and smaller than 75 µm (mean particle size: 37.5 µm) were obtained by sieving. Clinical formulation tablets (Tricor® Tablets, 80mg) were crushed using a mortar and pestle and used as the ASD formulation. Capsule formulations, containing 1 mg of FEN as an ASD formulation, were prepared by adding FEN ASD (crushed tablets) diluted with lactose at 10% (w/w for FEN) into size 9 gelatin capsules (PCCaps®) with a designated filling kit (Capsugel® Inc., Morristown, NJ, U.S.A.).

Dissolution Study  In vitro dissolution profiles of crystalline FEN and ASD formulation were evaluated using a small-scale dissolution vessel filled with fasted state simulated intestinal fluid (FaSSIF) stirred at 200rpm by a magnetic stirring system at 37°C. FaSSIF containing 0.75 mM lecithin and 3 mM taurocholic acid was adjusted to pH 6.5. Each dosage form of FEN (1 mg) was added to FaSSIF (8 mL). Then, 0.2 mL aliquots were collected at pre-determined time points. All samples were filtered through a polytetrafluoroethylene filter (Millex®-LH, pore size: 0.45 µm, Millipore, Billerica, MA, U.S.A.), and each filtrate (0.05 mL) was immediately mixed with 0.45 mL of a solution consisting of water and acetonitrile (50/50).

Animal Study  The ethics review committee at Setsunan University approved all animal experiments. Male Sprague–Dawley (SD) rats (weighing 250–300 g) were deprived of food but allowed free access to water for 18 h before the experiments. Crystalline FEN, with a mean particle size of 400 µm or 37.5 µm, was suspended in 0.5% (w/v) methylcellulose solution at a concentration of 0.5, 1, or 2 mg/mL. Each suspension (0.5, 1, or 2 mL) was administered to rats via a feeding tube by syringe. Immediately after ASD formulation suspension in water at a concentration of 2 or 1 mg/mL, 0.5 or 1 mL, respectively, was administered to rats. Capsules were orally administered using a dosing syringe. Subsequently, 0.5, 1, or 2 mL of water was ingested via feeding needle. For all in vivo experiments, blood samples (300 µL) were collected from the jugular vein at pre-determined time points. Blood samples were centrifuged and collected plasma was stored at −30°C before quantification. The area under the plasma concentration-time curve of FA (AUC₀⁻₁₂) at time 0 to 12 h after the oral administration of FEN was calculated in accordance with the trapezoidal rule.

Analytical Method  Samples from in vitro dissolution experiments were analyzed by reversed-phase HPLC (LC-10A Shimadzu Co., Kyoto, Japan) equipped with a variable wavelength UV detector (SPD-10A, Shimadzu Co., Kyoto, Japan). A J’sphere ODS-H80 (4.6 × 75 mm, YMC, Japan) was used with a mobile phase consisting of 0.1% (v/v) phosphate (solvent A) and acetonitrile (solvent B). The mobile phase ratio (solvent A/solvent B) was set to 80/20. The flow rate was 1.0 mL/min. FEN was quantified using the variable wavelength UV detector at 288 nm.

All plasma samples (0.1 mL) were mixed with 0.9 mL of acetonitrile. The mixture was shaken, and the supernatant (0.8 mL) collected by centrifugation. After solvent removal under vacuum at 40°C, the residue was dissolved in 0.1 mL of the solution consisting of 0.1% (v/v) formic acid and acetonitrile (50/50). The amount of FA in the treated solution was measured using a ultra performance liquid chromatography (UPLC) system (ACQUITY® UPLC, Waters, MA, U.S.A.) equipped with a tandem mass spectrometer (ACQUITY® TQD, Waters). A reverse-phase column (Waters Acquity® UPLC BEH C18 column, 2.1 × 50 mm, Waters) was used with a mobile phase consisting of 0.1% (v/v) formic acid in water (solvent A) and acetonitrile containing 0.1% (v/v) formic acid (solvent B) with a gradient of 40°C. The initial mobile phase was 98% solvent A and 2% solvent B pumped at a flow rate of 0.3 mL/min. Between 0 and 1.0 min, the percentage of solvent B increased linearly to 95%, at which it was maintained for 1.0 min. Between 2.01 and 2.5 min, the percentage of solvent B decreased linearly to 2%. This condition was maintained until 3 min, at which time the next sample was injected into the UPLC system. All treated samples were injected at a volume of 10 µL into the UPLC system. The monitored transition of FA was set at m/z 319.04→233.06 (positive ion mode).

Statistical Analysis  All data are presented as means with the standard deviation (S.D.) for individual groups. Significance was assessed by the unpaired t-test and p-values of 0.05 or less were considered significant.

RESULTS

In Vitro Dissolution of FEN from Crystalline and ASD Formulations  The dissolved concentration of FEN from crystalline in FaSSIF linearly increased with time, irrespective of the particle size (Fig. 1). Although the difference in the particle size was approximately 10-fold, 37.5 and 400 µm, the difference in the dissolution rate, the slope between dissolution concentration and time, was only approximately 2-fold.

![Fig. 1. Effect of Particle Size, 400 µm (Square Symbol) and 37.5 µm (Circle Symbol), and ASD Formulation (Triangle Symbol) on FEN Dissolution in FaSSIF](image-url)
In comparison, FEN dissolution was significantly enhanced by amorphous form (Fig. 1). However, after 5 min, the dissolved concentration in FaSSIF reached a maximum value (27.1 ± 6.8 µg/mL) then dramatically decreased with time, indicating the supersaturation of FEN and subsequent rapid precipitation.

**Effects of the Particle Size and Ingested Water Volume on FEN Suspension Absorption** The plasma profiles of crystalline FA were markedly influenced by particle size and ingested water volume (Fig. 2, Table 1). The oral absorption of smaller particle size FEN after administration of 0.5 mL water was 2-fold greater than the larger particle size despite it not being statistically significant. A similar tendency was observed when the ingested water volume was increased. Furthermore, the increase in ingested water volume significantly enhanced FEN oral absorption irrespective of the particle size.

**Effect of Crystalline FEN with Small Particle Size on Dose Strength** To clarify the rate-limiting step of FEN oral absorption, the dose-escalation study was performed with small particle size crystalline FEN without change in ingested water volume (Fig. 3). The increase in the dose strength of FEN dramatically enhanced oral absorption ($C_{\text{max}}$ and $AUC_{0-12}$). The systemic exposure of FA linearly increased in the increase of the dose strength ranging from 0.5 mg/rat to 2 mg/rat.

**Effects on the Oral Absorption of ASD Formulation FEN by Ingested Water Volume and Dosage Forms** Figure 4a shows the plasma profile of FA after oral administration of FEN ASD immediately after suspension in water (0.5 and 1 mL). The oral absorption of FEN was not influenced by the ingested water volume (Table 1). However, FEN absorption of the amorphous formulation was approximately 4-fold that of the small particle size crystalline FEN administered with 1 mL of water. When FEN ASD was administered as a solid using a gelatin capsule, the plasma profile of FA showed a large inter-individual difference (Fig. 4b). However, the $AUC_{0-12}$ of FA after administration with 1 or 2 mL of water was comparable to that from suspended ASD formulation (Table 1).

**DISCUSSION**

The rate-limiting step in the oral absorption of BCS class II drugs is generally either dissolution rate or solubility. Oral
absorption of crystalline FEN in humans was significantly increased by particle size reduction, suggesting that the rate-limiting step is dissolution. Therefore, the same limiting step in oral crystalline FEN absorption should be detected in rats. As dose strength may alter these limiting steps, it was carefully selected. In clinical treatment with FEN, 200 mg is generally used for humans in a micronized formulation. Thus, a similar ratio of body weight to dose was used for the rats (1 mg) in this study. Particle size reduction significantly improved in vitro dissolution and in vivo oral absorption of FEN (Figs. 1, 2, Table 1). The in vivo observations corresponded well with in vitro dissolution study. The systemic exposure of FA (AUC0-12) linearly increased with the increase in the FEN oral dose (Fig. 3, Table 1). This demonstrates that, at doses examined in this study, dissolution is the rate-limiting step of FEN absorption, consistent with that in humans. The increasing ingested water volume dramatically enhanced the FEN oral absorption. As oral drug absorption is simply determined by the relationship between the effective area and dissolved drug concentration at the site of absorption, it is expected that a difference in the ingested water volume influences the drug absorptive area and/or the effective fluid volume for drug dissolution. Tanaka et al. investigated the luminal concentration-time course of fluorescence dextran-4000 (FD-4), a nonabsorbable compound with high solubility, at each segment of the GI tract after the ingestion of different volumes of water (0.5 and 1 mL). Although the stomach fluid volume strongly depends on the ingested water volume as well as the clinical study, the fluid volume in each intestinal tract segment is not influenced by water volume. Furthermore, the oral absorption of atenolol, a poorly permeable drug with high solubility, was not influenced by differences in ingested water volume. Similar results were observed in our previous study. These findings indicate that the drug absorption effective area was not influenced by ingested water volume.

Many reports suggest that nano- to micro-scale drug particles can exist in the unstirred water layer (UWL) on the intestinal epithelium surface, and these particles induced a reduction in the effective thickness of the UWL. Assuming that the effective fluid volume for FEN dissolution was not influenced by differences in ingested water volume, the observed effect of the ingested water volume on FEN oral absorption could not be explained only by a reduction in UWL thickness, irrespective of particle size. Orally ingested water can be absorbed from the intestine via a passive process promoted by the osmotic gradients and aquaporins. After the movement of the gastric fluid to the small intestine, excess water is rapidly absorbed, and the fluid volume reaches a steady state. Therefore, the absorption rate of the ingested water in the small intestine after ingestion of a larger volume could be relatively rapid compared to that of a smaller volume. When both factors, water absorption and particle drifting, are considered, the effective thickness of the UWL may be reduced by the increase in the ingested water volume. Therefore, a larger volume of ingested water may further enhance the oral absorption of FEN, irrespective of the particle size. However, this requires further study to evaluate.

Supersaturation-generating formulations, such as ASD and lipid-based formulations, significantly improve the oral absorption of BCS class II drugs, including FEN. FEN absorption was improved by the ASD technique and so ASD formulations are currently used as clinical formulations in Japan. Many in vivo studies during preclinical study adopt a suspension form for oral administration. We observed, during in vitro dissolution, FEN supersaturation evidenced by

![Fig. 3. Plasma Concentration Profile of FA after FEN Oral Administration at a Dose of 0.5 mg/rat (Triangle Symbol) or 2 mg/rat (Square Symbol) Together with That of 1 mg/rat (Circle Symbol). Data are expressed as the mean ± S.D. of at least three independent experiments.](image)

![Fig. 4. Effect of the Ingested Water Volume, 0.5 mL/rat (Open Symbol with Solid Line), 1 mL/rat (Closed Symbol with Solid Line), and 2 mL/rat (Closed Symbol with Broken Line) and Dosing Form, Suspension Form (a) and Solid Form (b), on the FEN Oral Absorption of a Clinical ASD Formulation at a Dose of 1 mg/rat. Data are expressed as the mean ± S.D. of at least three independent experiments.](image)
the dissolution of the clinical ASD formulation of FEN (Tricor® Tablets) followed by a rapid decrease in concentration, indicating precipitation (Fig. 1). When the suspension of ASD formulation was administered to rats, the oral absorption was markedly enhanced compared to the crystalline form (Fig. 4a and Table 1). In addition, the plasma profile of FA administered FEN with 0.5 mL of water was comparable to that with 1 mL of water. These results showed the ASD formulation was superior to the micronized formulation. Although a suspension form can be convenient and reduce the inter-individual difference, the absorption process, especially for supersaturable formulations, is not consistent with the in vivo absorption process. Therefore, in our previous studies, the oral administration study with rats was performed with a gelatin capsule containing solid formulations such as ASDs and cocrystals to evaluate the in vivo absorption processes. When the ASD formulation, encapsulated in a gelatin capsule, was administered with water to rats, there were large individual differences in plasma profile, independent of ingested water volume (Fig. 4b, Table 1). Oral absorption of FEN from a solid ASD form with 1 or 2 mL of water was comparable to that of the suspended ASD formulation; however, the $AUC_{0-12}$ and the maximum plasma concentration ($C_{\text{max}}$) of FA with 0.5 mL of water were relatively less than those with larger volumes of water (Table 1). This suggested that the ingested water volume markedly affects the in vivo performance of ASD formulations administered as a solid form. Our previous study revealed that the ingested water volume influences formulation disintegration, drug dissolution rate, and gastric emptying in rats after oral administration of solid forms, mini-tablets, and capsules. The absorption rate of BCS class I drugs significantly decreased when the water administration volume was reduced, although the increased volume had no effect on the absorbed amount. One or two milliliters of water for ASD capsule formulation ingestion produced a similar availability as ASD administered as a suspension, whereas the plasma concentration of FA during the initial phase was lower than for the suspended ASD form. This slower absorption may be attributed to the time required for capsule disintegration and gastric emptying. As it is recommended to take a medicine with a glass of water (240 or 250 mL) in humans, the volume and weight ratio used (water volume: 0.5 mL, body weight: 250–300 g) in rats is approximately half of that in humans (water volume: 240–250 mL, body weight: 60–70 kg). Thus, the small volume of ingested water for rats was not suitable to evaluate oral absorption for an oral drug administration study. One milliliter of water is the minimum required to accurately evaluate of in vivo performance of such formulations. To demonstrate a suitable water volume for oral administration study with rats, further in vivo study of various drugs with poor water-solubility and formulations is needed and will be the subject of future study.

In the present study, the ingested water volume dramatically influenced oral FEN absorption. This change is anticipated for other species, including humans. Therefore, the influence of ingested water volume on the oral absorption of poorly water-soluble drugs and formulation effects must be assessed with other species. If a similar effect due to ingested water volume is observed in humans, then this study will aid in effective formulation development for humans.

CONCLUSION

In this study, we investigated, in rats, the effect of ingested water volume on the oral absorption of FEN, a BCS class II drug, from micronized and ASD formulations. The rate-limiting step in oral absorption of crystalline FEN was the dissolution process, which is the same in humans. We revealed that the ingested water significantly affects the in vivo performance of crystalline and ASD formulations of FEN. Therefore, the ingested water volume should be carefully set to evaluate the oral absorption of poorly water-soluble drugs. We recommend that oral administration studies with rats (250–300 g) be performed with at least 1 mL of ingested water to evaluate the in vivo performance of solid formulations of poorly water-soluble drugs.

Conflict of Interest The authors declare no conflict of interest.

REFERENCES

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