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Quantitative Analysis of the Effect of Supersaturation on in Vivo Drug Absorption

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Abstract: The purpose of this study is to clarify the effects of intestinal drug supersaturation on solubility-limited nonlinear absorption. Oral absorption of a novel farnesyltransferase inhibitor (FTI-2600) from its crystalline free base and its HCl salt was determined in dogs. To clarify the contribution of supersaturation on improving drug absorption, in vivo intraluminal concentration of FTI-2600 after oral administration was estimated from the pharmacokinetics data using a physiologically based model. Dissolution and precipitation characteristics of FTI-2600 in a biorelevant media were investigated in vitro using a miniscale dissolution test and powder X-ray diffraction analysis. In the in vitro study, the HCl salt immediately dissolved but precipitated rapidly. The metastable amorphous free base precipitant, which did not convert into the stable crystalline free base in the simulated intestinal fluids for several hours, generated a 5-fold increase in dissolved concentration compared to the equilibrium solubility of the crystalline free base. By computer simulation, the intraluminal drug concentration after administration of the free base was estimated to reach the saturated solubility, indicating solubility-limited absorption. On the other hand, administration of the HCl salt resulted in an increased intraluminal concentration and the plasma concentration was 400% greater than that after administration of the free base. This in vivo/in vitro correlation of the increased drug concentrations in the small intestine provide clear evidence that not only the increase in the dissolution rate, but also the supersaturation phenomenon, improved the solubility-limited absorption of FTI-2600. These results indicate that formulation technologies that can induce supersaturation may be of great assistance to the successful development of poorly water-soluble drugs.

Keywords: Oral absorption; supersaturation; solubility; in vivo/in vitro correlation (IVIVC); crystalline salt; solubility-limited absorption

Introduction

The number of poorly water-soluble drug candidates in drug discovery has recently increased.[1] Low solubility often results in nonlinear and dose-dependent oral absorption; that is, the fraction of dose absorbed (F_a) decreases as the dose increases.[2,3] Insufficient exposure due to the nonlinear absorption often makes it difficult to evaluate the efficacy and safety of a drug in both the preclinical and clinical stages of drug development. One of the causes of nonlinear absorption is the solubility-limited absorption in which in vivo dissolution of a drug in the GI tract occurs under nonsink conditions; thus, an increase in the dissolution rate or the dose does not lead to an increase in the amount absorbed.[4] Avoiding nonlinear absorption is an issue of great

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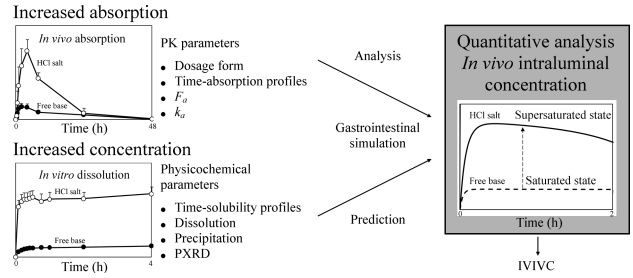
interest but is a difficult challenge because traditional formulation technologies mainly improve the dissolution rate of drugs, but not the solubility.

Generating supersaturation can improve the solubility of drugs[5] because drugs in a state of supersaturation are kinetically soluble in solution at a concentration above their thermodynamic equilibrium solubility. In this article, supersaturation is defined as a concentration which is higher than the equilibrium solubility of the solid form. If a supersaturated drug solution exists in the gastrointestinal lumen for a sufficient length of time to be absorbed, it may result in an enhanced flux across the intestinal wall and thus improve nonlinear absorption. Therefore, supersaturation would be a powerful aid in successfully developing poorly water-soluble drugs.

Several formulations that induce supersaturation in vitro and enhance oral absorption in vivo include amorphous materials such as solid dispersion,[6−8] crystalline salts,[9] cocrystals,[10,11] higher energy polymorphic forms,[12] and SEDDS.[13] However, it is still difficult to evaluate quantitatively what effect supersaturation had on the enhanced absorption because these formulations often increase not only the dissolved concentration but also the dissolution rate of drugs. Also, *in vitro/in vivo* correlation of supersaturable formulation has not yet been understood clearly.

In this study, a crystalline salt which induced supersaturation in vitro was orally administered to dogs in vivo. To prove the contribution of supersaturation to improved oral absorption, the kinetics of the in vivo intraluminal drug concentration was estimated from the plasma concentration data using a physiologically based model.[14] The dissolved concentration and solid state analysis of the precipitant from the crystalline salt were also investigated in vitro. The strategy in this study to consider the correlations of increased absorption and intraluminal concentrations in vivo and the increased concentration in vitro is shown in Scheme 1.

Scheme 1



A novel, highly potent and selective farnesyltransferase inhibitor (FTI-2600) has been developed for the treatment of several types of tumors.[15] The structure of FTI-2600 is shown in Figure 1. Free base FTI-2600 is insoluble in water and in buffers at neutral pH and has a molecular weight of 447.5 g/mol, a high log *P* value (ClogP: 3.18), and basic functional groups (pK_a: 2.9, 5.1, both bases). Because the permeability is high (Caco-2 permeability: 3.2×10^{-5} cm/s), FTI-2600 is classified as a weakly basic BCS class II drug. Weakly basic drugs are likely to primarily dissolve in the stomach due to the low gastric pH. After dissolution in the stomach, transfer to the intestine may result in supersaturated luminal concentrations that may increase the absorption.[16] However, in the case of an elevated gastric pH (e.g., achlorhydria, patients receiving gastric acid blockers, and some pathological conditions), gastric dissolution and subsequent intestinal supersaturation of weakly basic drugs is inhibited[17,18] and may result in insufficient and variable absorption.

Because the dose-to-solubility ratio of FTI-2600 is very high (more than 10 L) at the higher anticipated human doses (600−1200 mg),[19] solubility-limited and nonlinear absorption was predicted for subjects with elevated gastric pH levels.[4] If the dissolution of FTI-2600 in the gastric fluid of high pH patients was improved, oral absorption would be kept high regardless of the pH ranges of gastric fluid and thus the variance in absorption could be minimized. Therefore, salt screening was carried out to improve the physicochemical and biopharmaceutical properties and resulted in an HCl crystalline salt of FTI-2600. The characterization and pharmacokinetics of the FTI-2600 HCl salt are described herein.

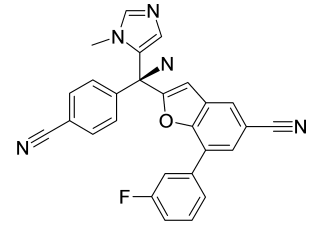


Figure 1. Structure of FTI2600.

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Materials and Methods

Materials. The free base of FTI-2600 was synthesized in-house. An intravenous formulation of famotidine, Gaster10, was purchased from Astellas Pharma Inc. (Tokyo, Japan). HCO60 was purchased from Kao Corporation (Tokyo, Japan). Sodium taurocholate was purchased from Wako Pure Chemical Industries (Osaka, Japan). L-α-Phosphatidylcholine was purchased from Nippon Oil and Fats Corporation (Tokyo, Japan). All chemicals used were of standard research grade.

Preparation of the HCl Salt and the Free Base. The crystalline free base (melting point 161 °C) was prepared as follows. FTI-2600 (0.1 g) was dissolved in acetone (0.5 mL) at ambient temperature. Cyclohexane (1 mL) was added, and the resulting solution was stored at ambient temperature for 2 days. Crystals were isolated by filtration and allowed to dry under vacuum. The obtained crystals had a volume mean diameter of 1 μm. Before the in vitro and in vivo studies, the free base particles were suspended in 5% arabic gum aqueous solution at 5 mg/mL.

The crystalline HCl salt (melting point 241 °C) was prepared as follows. FTI-2600 (0.3 g) was dissolved in ethanol (3 mL) at ambient temperature. A 5 M hydrochloride solution (0.27 mL) was added and the resulting solution was stored at ambient temperature for 3 h. Crystals were isolated by filtration and allowed to dry under vacuum. Sieve fractions of 40−60 μm were obtained. Before the in vitro and in vivo studies, the HCl salt was mixed with an equal amount of lactose as an excipient to improve the wettability of the drug.

Particle size analysis included examination using series VH-8000 and VH-Z450 microscopy (Keyence, Osaka, Japan) and Image-Pro Plus 5.1J analysis software (Media Cybernetics, Bethesda, MD) to measure the Feret diameter.

Melting points were determined using a 10002 hot stage (Japan High Tech, Tokyo, Japan) and OPTIPHOT2-POL microscopy (Nikon, Tokyo, Japan). The samples were heated from 25 to 350 °C at a rate of 10 °C/min.

Powder X-ray Diffraction (PXRD). PXRD patterns in the range of 3° to 35° 2θ were obtained using an X'Part PRO MPD powder X-ray diffractometer (Spectris, Tokyo, Japan) using Cu Kα radiation in reflectance mode. Tube voltage and amperage at 45 kV and 40 mA, respectively, were used. Divergence and scattering slits were set at 0.5°, and the receiving slit was set at 0.2 mm. The samples were packed in an aluminum sample holder and measured with a continuous scan at 1.2° 2θ/min with a step size of 0.02° 2θ.

Miniscale Dissolution Test. Dissolution rate and time profiles of solubility were measured using a miniscale dissolution test as previously reported.[14] The miniscale dissolution tests were carried out by the paddle method (50 rpm, 50 mL) using a VK7010 dissolution station and a VK8000 dissolution sampling station (Varian Medical Systems, Inc., Palo Alto, CA) in a 100 mL glass vessel (42 mm diameter × 105 mm) (Takao Manufacturing Co., Ltd., Kyoto, Japan). Ten milligrams of drug as a free base was added to 50 mL of dissolution medium for the test to determine the supersaturation of the drug. $FaSSIF_{dog}$, a physiologically biorelevant medium in dogs containing 5 mM sodium taurocholate and 1.25 mM lecithin in 29 mM of phosphate buffer (pH 6.5),[4] was used for the dissolution medium. Form analysis measured by PXRD was carried out after the isolation of the solids in the vessels. To simulate dissolution in the GI tract, the precipitant from the HCl salt in a phosphate buffer (pH6.5) without bile salt and lecithin, which simulate the gastric fluids of a pH-elevated patient, was added to $FaSSIF_{dog}$. The dissolution rate and solubility of the precipitant in $FaSSIF_{dog}$ were then determined.

Equilibrium Solubility Study. The equilibrium solubility of a crystalline free base was determined after 24 h of equilibration in media at 37 °C using a shaking incubator as previously reported.[4] A 96-well polypropylene plate containing 0.5 mL of medium per well was placed in the incubator. Excess amounts of drugs were then added to the wells; the experiments were carried out in triplicate. Phosphate buffers (pH 6.5) containing 0−20 mM sodium taurocholate and 0−5 mM lecithin were used for the solubility study. After 24 h, the aqueous samples were filtered through a MultiScreen solubility plate with a 0.4 μm polycarbonate isopore membrane (Millipore Corporation, Billerica, MA) and the pH of the filtrate was measured. The first 0.2 mL was discarded to avoid loss of drug from the sample due to adsorption. The remainder of the sample was diluted with an equal volume of tetrahydrofuran, including 0.8 mg/mL of *p*-hydroxybenzoic acid *n*-dodecyl ester, as an internal standard (IS).

High-Performance Liquid Chromatography Analysis of the Miniscale Dissolution Test and the Equilibrium Solubility Study. Sample concentrations for the dissolution test and the equilibrium solubility study were determined by Waters 2795 separation module HPLC using a Waters 2487 dual λ UV/vis detector (Waters, Milford, MA). Diluted samples (10 μL) were injected onto a Cadenza CD-C18 3 μm 3.0 × 50 mm column (Imtakt Corporation, Kyoto, Japan). FTI-2600 was eluted with a mobile phase of water−acetonitrile−trifluoroacetic acid at a ratio (by volume) of 60:40:0.1, followed by a mobile ratio of 5:95:0.1 to elute *p*-hydroxybenzoic acid *n*-dodecyl ester as an internal standard and quantified with variable UV detection at 231 and 270 nm for the drug and *p*-hydroxybenzoic acid *n*-dodecyl ester, respectively. A standard curve was prepared and linearity

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was observed at a concentration range of approximately 0.02−80 μg/mL on a log−log plot (correlation coefficient of $r^2 > 0.999$) by linear regression analysis using Microsoft Excel 2000 (Redmond, WA).

In Vivo Oral and Intravenous Administration Study in Beagle Dogs. The drug FTI-2600 was administered orally to five beagle dogs (male, body weight 12−15 kg). A washout period of 1 week was maintained between consecutive administrations. The dogs were fasted and water intake restricted overnight and for 8 h postdosing. Following oral dosing, dogs were given 4 mL/kg of water. All procedures using animals were conducted in accordance with the ethical guidelines for animal care promulgated by Chugai Pharmaceutical Co, Ltd., and all experimental protocols were approved by the Institutional Animal Care and Use Committee in Chugai.

The suspension of the free base (3 mg/mL in a solution of 5% arabic gum) or the HCl salt mixed with lactose in capsules was orally administered at a dose of 3 mg/kg as a free base. An aqueous FTI-2600 solution, in which FTI-2600 was dissolved completely in a 10% HCO60 solution, was also orally administered at a dose of 3 mg/4 mL/kg. A FTI-2600 solution consisting of 2 equivalents of HCl in saline (pH 3) was intravenously administered at a dose of 3 mg/1 mL/kg. The pharmacokinetics data after intravenous administration were used to obtain either the bioavailability (BA) value using a noncompartmental analysis or the time profiles of BA using a deconvolution technique.

The achlorhydric dog model was used to prevent gastric dissolution and subsequent intestinal supersaturation of FTI-2600 due to a low gastric pH. To maintain a high gastric pH, dogs were intravenously treated with H_2-blocker famotidine (10 mg/mL/dog) 2 h before the FTI-2600 administrations.[20] The mean gastric pH induced by H_2-blocker 1 h after intravenous administration was 7.5 with a range of 7.0−8.6, maintained consistently for 4 h.[21] Blood samples (1 mL) were collected from a foreleg vein with a heparinized syringe at 0 (predose), 0.33, 0.67, 1, 2, 4, 8, 24, and 48 h after oral administration and 0 (predose), 0.08, 0.33, 0.67, 1, 2, 4, 8, 24, and 48 h after intravenous administration. Plasma samples were obtained from centrifugation of the blood samples and stored at −20 °C until use.

Analysis of Plasma Concentration. Plasma concentration was quantified by HPLC mass spectroscopy (LC−MS/MS). Dog plasma was spiked with FTI-2600 in MeOH to yield concentrations for a standard curve. To 50 μL of plasma standard and unknowns, 50 μL of MeOH and 50 μL of 0.004% acetonitrile aqueous solution including 0.1 μg/l of prazosin as IS were added. The samples were extracted using an Oasis μElution plate HLB solid phase extraction kit (SPE cartridges) (Waters). The concentration of FTI-2600 in plasma was determined by LC−MS/MS using an Alliance 2750 system separation module (Waters) connected to an Micromass Quattro micro LC-MS/MS system (Waters) with a C18 column (XterraC18 MS 2.5 μm 2.1 × 20 mm) (Waters). Elution was accomplished using a linear gradient that consisted of ramping water−acetonitrile−1% formic acid at a ratio from 90:5:5 to 5:95:5 delivered at 0.2 mL/min. The injection volume was 20 μL. The MS/MS instrument was operated in electrospray ionization (ESI) mode. Detection was performed in the positive-ion mode using SRM of *m/z* 448.87−82.96 and *m/z* 309.52−163.07 transitions for FTI-2600 and the IS, respectively. The linear regression analysis (weighted 1/concentration^2) showed correlation coefficients of linearity ($r^2 > 0.99$) at concentration ranges of 1−100 and 100−2500 ng/mL.

PK Analysis. The pharmacokinetic parameters were determined using the noncompartmental analysis module in WinNonlin,4.0.1 (Pharsight Corporation, Mountain View, CA). The AUC was calculated from 0 to infinity using a linear trapezoidal rule.

Deconvolution Analysis To Determine Time Profiles of F_a and k_a in Dogs. The in vivo absorption−time profiles of drugs were estimated using a numerical deconvolution technique that is used in the assessment of drug release and drug absorption from orally administered drug formulation.[22] The mean plasma concentration data from the oral administration study was designated the response function, and data from the intravenous administration study was designated the weight function. The deconvolution approach provides information about the kinetics of absorption throughout the entire intestine. The drug absorption rate k_a after oral administration of the solution was obtained by calculating input rates. After calculating the BA−time profiles of all oral administrations, the BA value at each time point for solid particles was divided by that of the solution administration at 48 h to obtain the relative BA time profiles of the solid administrations.

The relative BA of a solid dosage form and a solution orally administered (relative $BA_{solid/solution}$) was utilized as an alternative to F_a. The relative $BA_{solid/solution}$ for lipophilic drugs is almost equal to the F_a of the solid dosage form ($F_{a,solid}$). The relative BA was calculated as the ratio of the area under the curve for solid dosages (AUC_{solid}) to solution dosages ($AUC_{solution}$) where AUC_{solid} is the product of $F_{a,solid}$, $F_{g,solid}$, and $F_{h,solid}$ and $AUC_{solution}$ is the product of $F_{a,solution}$, $F_{g,solution}$, and $F_{h,solution}$ (subscript notations indicate the dosage form). F_g and F_h are the fractions of the dose-escaping metabolism by the GI mucosa and by the liver, respectively.

$$\text{relative } BA_{solid/solution} = \frac{AUC_{solid}}{AUC_{solution}} \times 100 = \frac{F_{a,solid} \times F_{g,solid} \times F_{h,solid}}{F_{a,solution} \times F_{g,solution} \times F_{h,solution}} \times 100$$

Assuming linear kinetics of drug metabolism from either administration, the relative BA represents the ratio of $F_{a,solid}$

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to $F_{a,solution}$. The numerical value of $F_{a,solution}$ for lipophilic drugs is assumed to be 1 because the drugs are administered as a solution and complete absorption is possible due to their high permeability.

$$F_{a,solid} = \text{relative BA}_{solid/solution}$$

Physiologically-Based Model for Drug Absorption. Drug dissolution and absorption in the GI tract in dogs were simulated using a physiologically based model. Equations in the present model were reported previously although the way to obtain parameters was different.[4,14] In the present study, the equilibrium solubility ($C_{s,vivo}$) in the small intestine was considered to be an unknown parameter because the purpose of this study is to analyze the intraluminal concentration in vivo. So $C_{s,vivo}$ was estimated from the time profiles of F_a in vivo in the present study but not from the dissolved concentration in the dissolution test. In addition, the actual in vivo absorption rate (k_a), obtained by the deconvolution technique, was applied in the model. Mass balances of solid and of dissolved drugs in the GI tract are given by the following equations:

$$\frac{dX_{s,vivo}(t)}{dt} = -zX_{0,s,vivo}\left(\frac{X_{s,vivo}(t)}{X_{0,s,vivo}}\right)^{2/3}\left(\frac{X_{s,vivo}(t)}{X_{0,s,vivo}}\right)^{-1/3} \times \left(C_{s,vivo} - \frac{X_{d,vivo}(t)}{V_{vivo}}\right)$$
$$= -zX_{0,s,vivo}\left(\frac{X_{s,vivo}(t)}{X_{0,s,vivo}}\right)^{1/3}\left(C_{s,vivo} - \frac{X_{d,vivo}(t)}{V_{vivo}}\right) \quad (1)$$

$$\frac{dX_{d,vivo}(t)}{dt} = zX_{0,s,vivo}\left(\frac{X_{s,vivo}(t)}{X_{0,s,vivo}}\right)^{1/3}\left(C_{s,vivo} - \frac{X_{d,vivo}(t)}{V_{vivo}}\right) - P_{UWL}S\frac{X_{d,vivo}(t)}{V_{vivo}}$$
$$= zX_{0,s,vivo}\left(\frac{X_{s,vivo}(t)}{X_{0,s,vivo}}\right)^{1/3}\left(C_{s,vivo} - \frac{X_{d,vivo}(t)}{V_{vivo}}\right) - k_aX_{d,vivo}(t) \quad (2)$$

where $X_{s,vivo}(t)$ is the mass of solid drug in the small intestine at time t and $X_{d,vivo}(t)$ is the mass of dissolved drug in the small intestine at time t. V_{vivo} is the intestinal fluid volume. S is the effective intestinal surface area. $X_{0,s,vivo}$, which is a function of the number of particles in vivo, was set equal to the dose administered. The letter z represents the dissolution rate parameter determined from the expression $3D/\rho hr_0$, where D is the diffusion coefficient, ρ is the density of drug, h is the initial diffusion layer thickness, and r_0 is the initial particle radius. The z value is multiplied by $(X_{s,vivo}(t)/X_{0,s,vivo})^{-1/3}$, a formula modified from a previous paper which includes the assumption that the diffusion layer thickness in a particle changes with time.[23] The dissolution rate parameter z of the crystalline free base and the amorphous free base obtained from the dissolution test was used for the simulation after the administration of the crystalline free base and HCl salt, respectively.[14] For the administration of the HCl salt, the in vitro dissolution rate from the precipitant was used. Because all of the HCl salt immediately dissolved and precipitated as an amorphous free base in the simulated gastric fluid for high pH patient in vitro, we assumed that the HCl salt had transformed into the amorphous free base before entering the intestine in vivo. In the previous study, the calculated unstirred water layer (UWL) permeability (P_{UWL}) of a drug was used in order to predict the in vivo intestinal absorption rate. In the present study, because the actual in vivo absorption rate k_a was obtained by the deconvolution technique, this was applied to simulate oral absorption. The hybrid parameter of $P_{UWL}S/V_{vivo}$, which is equal to k_a, in eqs 2 and 3 was replaced. The rate of absorption is given by

$$\frac{dX_{a,vivo}(t)}{dt} = P_{UWL}S\frac{X_{d,vivo}(t)}{V_{vivo}} = k_aX_{d,vivo}(t) \quad (3)$$

where $X_{a,vivo}(t)$ is the mass of absorbed drug at time t.

The predicted fraction of the dose absorbed (F_a) is the ratio between $X_{0,s,vivo}$ and $X_{a,vivo}(t)$.

$$F_a(t) = \frac{X_{a,vivo}(t)}{X_{0,s,vivo}} \times 100 \quad (4)$$

The time profiles of F_a were simulated using eqs 1−4. The intestinal transit time which was estimated from time profiles of F_a was used in the simulation. The equilibrium solubility ($C_{s,vivo}$) was determined by fitting the time profiles of F_a to the observed data.

The plasma concentration is given by the drug amount in the body, $X_b(t)$, divided by the volume of distribution, assuming that the ratio of blood and plasma concentration is 1:

$$\frac{dX_b(t)}{dt} = k_aX_{d,vivo}(t)F_gF_h - k_{el}X_b(t) \quad (5)$$

where F_g and F_h are the fractions of the dose-escaping metabolism by the GI mucosa and by the liver, respectively, and k_{el} is the elimination rate from the body.

To obtain simulated profiles, the fourth Runge−Kutta method was used with Stella5.1.1 software (Cognitus Ltd., North Yorkshire, U.K.).

Intraluminal Concentration Analysis in Dog Using Physiologically-Based Model. Equilibrium solubility of the crystalline free base and supersaturation from the HCl salt in the small intestine in dogs were the unknown parameters. To determine them, the simulated time profiles of F_a were fitted to those observed using the least-squares methods. The observed time profiles were determined from the in vivo plasma concentrations by the deconvolution technique (see the section on deconvolution analysis). The simulation was carried out using eqs 1−4 of the physiologically based model (see the previous section). When the time profiles of F_a were fitted, the equilibrium solubility of the free base and

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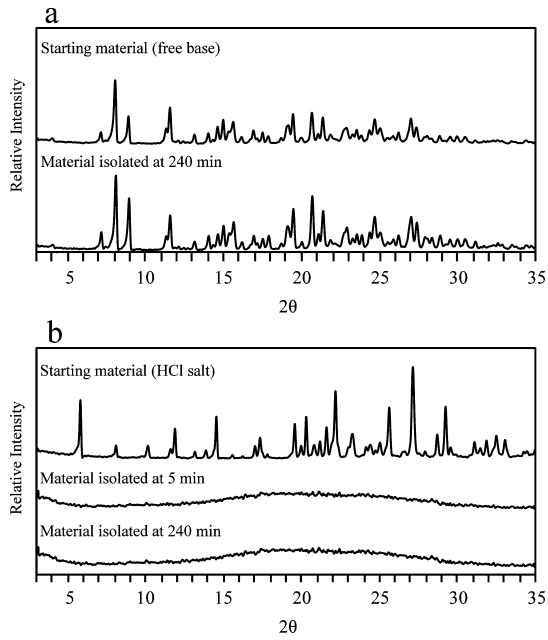

Figure 2. PXRD comparing FTI-2600 starting material and isolated material during the dissolution test. (a) Crystalline free base, (b) HCl salt.

supersaturation from the HCl salt in the small intestine in dogs were determined. The time profiles of intraluminal concentration ($X_{d,vivo}(t)/V_{vivo}$) were also obtained using eqs 1−3 when the simulated time profiles of F_a were fitted to the observed data. Then, the plasma concentration profiles simulated using eqs 1−5 were compared with those observed to confirm the reliability of the simulation.

Results

Miniscale Dissolution Test and PXRD Analysis of Precipitant during Dissolution Experiments. The dissolution profiles of the crystalline free base and the crystalline HCl salt of FTI-2600 in the miniscale dissolution test with $FaSSIF_{dog}$ are illustrated in Figure 3. The free base showed immediate release and solubility reached a plateau with a dissolution rate parameter z of 0.59 mL/mg/min and equilibrium solubility of 33 μg/mL. On the other hand, drug concentration reached 168 μg/mL in $FaSSIF_{dog}$ when HCl salt was added, and this concentration was maintained for >4 h. As shown in Figure 2, the precipitant from HCl salt in the amorphous form remained unchanged during the dissolution test. Because the concentration of 168 μg/mL is significantly greater than the equilibrium solubility (32 μg/mL) of the crystalline free base, it was considered that the amorphous free base generated from HCl salt might produced supersaturation compared to the equilibrium solubility of the crystalline free base. The concentration of 168 μg/mL might also be the equilibrium solubility of the amorphous free base. The isolated, amorphous precipitant reproduced the supersaturation in $FaSSIF_{dog}$, and the dissolution rate parameter z of the precipitant was 0.33 mL/mg/min. Table 1 summarizes the results of the dissolution and solubility tests. The equilibrium solubility of the crystalline free base increased

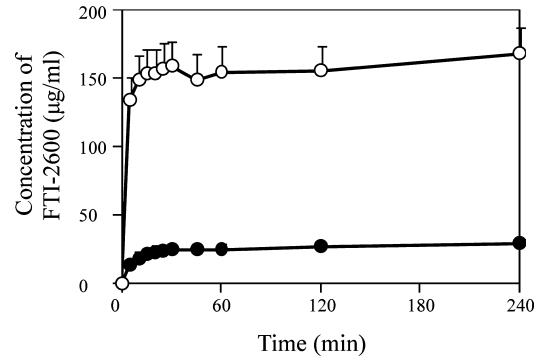

Figure 3. Dissolution and solubility of FTI-2600 crystalline free base (●) and the HCl salt (○) in $FaSSIF_{dog}$.

as the concentration of sodium taurocholate and lecithin in a medium (pH 6.5) was increased.

In Vivo Oral Administration Study in Dog. The mean plasma concentration−time profiles of FTI-2600 after oral dosing to dogs are shown in Figure 4. The HCl salt dramatically increased the systemic exposure of FTI-2600. The mean C_{max} and AUC_{0-inf} values were 4-fold greater than those for the free base (Table 2). The relative $BA_{solid/solution}$ values (calculated as an alternative to F_a) of the crystalline free base and the HCl salt were 28% and 109%, respectively. The BA after the administration of solution was 54%, which was estimated to be the F_gF_h value, assuming that the F_a of the solution administration is 1.

From the deconvolution analysis the BA at 4 h of the solution, the crystalline free base and the HCl salt was 57%, 12%, and 63%, respectively (Figure 5a). From the plasma concentration after administration of solution, the absorption rate of FTI-2600 was determined to be 1.4 ± 0.3 h^{-1}. The relative $BA_{solid/solution}$ (calculated as an alternative to F_a) at 4 h was 20% for the crystalline free base and 108% for the HCl salt (Figure 5b). The time periods of absorption for the free base and HCl salt were estimated to be 2 and 3 h, respectively. The results of deconvolution analysis corresponded well to those of noncompartmental analysis.

Estimation of in Vivo Intraluminal Drug Concentration in Dog. In order to evaluate the contribution of supersaturation on oral absorption of FTI-2600, in vivo intestinal drug concentration was estimated. By fitting the simulated profiles of F_a (Figure 6b) to those observed, the equilibrium solubility ($C_{s,vivo}$) in the small intestine in dogs after administrations of the crystalline free base and the HCl salt were determined to be 50 ± 14 and 244 ± 108 μg/mL, respectively. Then, time profiles of the intraluminal concentrations of FTI-2600 were obtained using eqs 1−3 (Figure 6c). In comparison, the intraluminal concentration ($X_{d,vivo}(t)/V_{vivo}$) after administration of the free base was approximately 47 μg/mL, which is almost the same as the $C_{s,vivo}$ value, suggesting that absorption is limited by the solubility. In the case of the HCl salt, the intraluminal concentration increased to 160−200 μg/mL, which was 4-fold higher than that from the free base. These results clearly indicated that the supersaturation also occurred in vivo in the small intestine

Table 1. The Equilibrium Solubility and the Dissolution Rate Parameter of FTI-2600 Crystalline Free Base, the Dissolved Concentration and the Dissolution Rate Parameter of FTI-2600 HCl Salt, and the PXRD Form of Materials Isolated after the Test[a]

buffer composition[b]		crystalline free base			HCl salt		
NaTC (mM)	lecithin (mM)	equilibrium solubility (μg/mL)	dissolution parameter z (mL/mg/min)	PXRD form	dissolved concn (μg/mL)[e]	dissolution parameter z (mL/mg/min)	PXRD form
0	0	3.7 ± 0.1		FB[c]			
3	0.75	21 ± 0.5		FB[c,d]			
5	1.25	32.6 ± 0.6	0.59 ± 0.07	FB[c]	168.1 ± 19.3	10>[f]	AM[d]
						0.33 ± 0.02[g]	AM[d]
15	3.75	121.9 ± 5		FB[c]			
20	5	205 ± 1.7		FB[c]			

[a] NaTC, sodium taurocholate; FB, crystalline free base; AM, amorphous free base. [b] Phosphate buffer (pH 6.5) with various concentrations of NaTC and lecithin. [c] Solid form isolated after the solubility study. [d] Solid form isolated after the dissolution study. [e] Mean concentration in the dissolution test at 4 h. [f] Dissolution rate of HCl salt. [g] Dissolution rate of the precipitant.

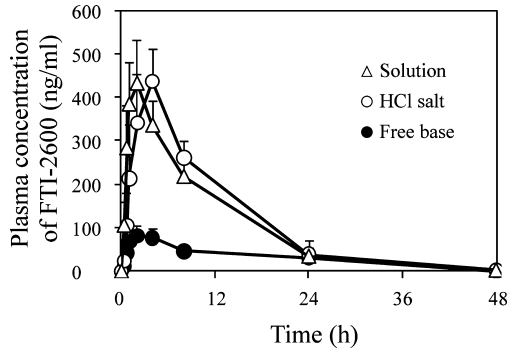

Figure 4. Mean (±SD) plasma concentrations of FTI-2600 following oral administration in beagle dogs (n = 5) under fasted conditions at a dose of 3 mg/kg. (△) FTI-2600 crystalline free base dissolved in 10% HCO60 solution, (○) a mixture of FTI-2600 HCl salt and lactose encapsulated in hard gelatin capsules, (●) FTI-2600 crystalline free base suspended in 5% arabic gum.

and may contribute to improved oral absorption of FTI-2600. Time profiles of the plasma concentration simulated with the determined parameters in Table 3 accorded well with the observed profiles (Figure 6a), confirming the adequacy of the absorption model used in this study.

Discussion

When nonlinear solu bility-limited absorption is observed or predicted for a drug candidate,[2,4] preformulation or front-loaded formulation studies to improve solubility should be launched as early as possible to minimize the risk of inadequate exposure.[24,25] In the present study, through a computational analysis of in vivo intraluminal drug concentration and an in vivo/in vitro correlation of the increased concentration generated by the FTI-2600 HCl salt, we found that the increased dissolved concentration, but not the dissolution rate, contributed to the improved oral absorption of a poorly water-soluble drug. Present results clearly demonstrate the possibility of improving the solubility-limited nonlinear absorption of drugs by utilizing formulation technologies which target supersaturation.

Crystalline salts have been applied mainly to improve dissolution rate-limited absorption[26−29] because salt formation can increase the dissolution rate due to the change in the solid surface pH.[30−32] The present study shows that a crystalline salt can also improve solubility-limited absorption if the precipitant in the suspension is in a metastable solid. The HCl salt of FTI-2600 immediately dissolved but precipitated in buffers at pH 6.5. The precipitant was a metastable amorphous free base and reproduced supersaturation for more than 4 h. The increased concentration may be the equilibrium solubility of the amorphous free base but can also be said to be supersaturation compared to the equilibrium solubility of the crystalline free base. In the in vivo study in dogs, supersaturation from the HCl salt, which had high concentration compared to the equilibrium solubility of the crystalline free base, clearly contributed to the improved absorption. These results verified that in vitro characteristics reflect in vivo dissolution profiles in the GI tract and, thus, the effect of supersaturation on oral drug absorption can be estimated from an in vitro study when a metastable solid such as an amorphous free acid/base precipitates out in the stomach and is then absorbed in the small intestine. If the stable crystalline free acid/base quickly

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Table 2. FTI-2600 Pharmacokinetic Parameters and Summarized Statistics Following Oral and Intravenous Administration to Beagle Dogs at a Dose of 3 mg/kg

	AUC_{inf} (ng/mL/h)	C_{max} (ng/mL)	t_{max} (h)	BA (%)	rel $BA_{solid/solution}$ (%)
iv[a]	8012 ± 1867				
soln in 10% HCO60 aqueous soln	4327 ± 996* [b]	444 ± 99*	1.6 ± 0.5	54	
crystalline free base	1236 ± 509	91 ± 15	8 ± 10.7	15	28
HCl	4717 ± 413*	437 ± 74*	4 ± 0	59	109

[a] Total clearance and volume distribution obtained were 390 ± 87 (mL/h/kg) and 3253 ± 659 (mL/kg), respectively. [b] (*) Significant difference ($P < 0.05$) compared with free base.

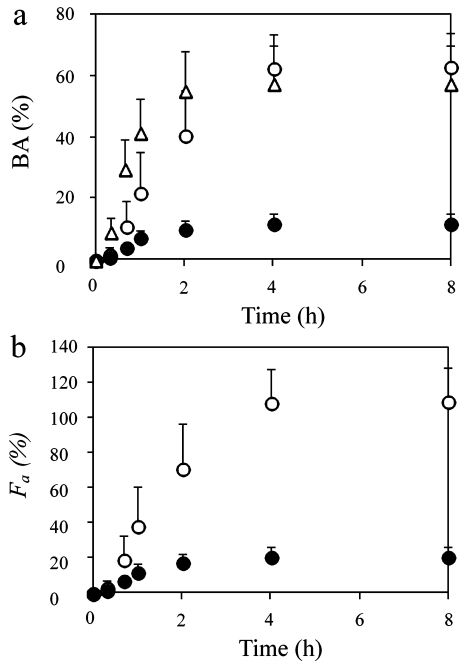

Figure 5. The in vivo absorption−time profiles of FTI-2600 after oral administration of (Δ) FTI-2600 solution, (○) the HCl salt, and (●) the crystalline free base. Results are expressed as the mean; bars indicate the SD value. (a) The mean BA−time profiles of all oral administrations determined using the numerical deconvolution technique. The deconvolution analysis was carried out using plasma concentration data from the oral administration study as the response function and data from the intravenous administration study as the weight function. (b) The mean F_a−time profiles after the administration of the HCl salt and the crystalline free base. The relative bioavailability of a solid dosage form and a solution orally administered served as the F_a.

precipitates from supersaturation, the supersaturation might be ineffective in improving solubility-limited absorption in vivo.

To consider the in vivo dissolution and precipitation from supersaturation, we have used biorelevant media in the in vitro assay. Composition and/or properties of the dissolution media have significant effects on the dissolution and precipitation of poorly water-soluble drugs. Because GI fluids include complex components, prediction of in vivo dissolution profiles from the in vitro study with simple buffer solution is difficult. Since Dressman et al. suggested that simulated GI fluids in humans can estimate the in vivo dissolution of drugs,[33−37] various studies have been carried out in this area.[38−40] Differences in the composition of the intestinal fluids of dogs and humans have been reported, including the concentrations of bile salts.[41] Both Dressman's group and our group noted that the concentration of the bile salt in simulated intestinal fluids in dogs (5 mM NaTC and 1.25 mM lecithin) was greater than that in humans (3 mM NaTC and 0.75 mM lecithin).[4,42,43] In the present study, the equilibrium solubility of FTI-2600 free base and the increased concentration from HCl salt in the small intestine calculated from plasma concentration data corresponds to those observed in vitro with 5 mM NaTC and 1.25 mM lecithin buffer (33 ± 0.6 μg/mL and 168 ± 19 μg/mL for the free base and the HCl salt, respectively). This result indicates that drug dissolution and precipitation from supersaturation in the intestine can be estimated from in vitro data when the simulated intestinal fluids contain an appropriate concentration of bile salts and lecithin.

Precipitation from a supersaturated solution is one of the important factors in developing a formulation to improve absorption. The nucleation rate and the crystal growth rate are the determinants of precipitation of a crystalline free acid/base. No crystalline free base of FTI-2600 was observed from the amorphous free base for at least 4 h in the dissolution test, indicating that the nucleation rate of crystalline free base was slow. According to the theory of nucleation, the rate of nucleation depends on the degree of supersaturation, the

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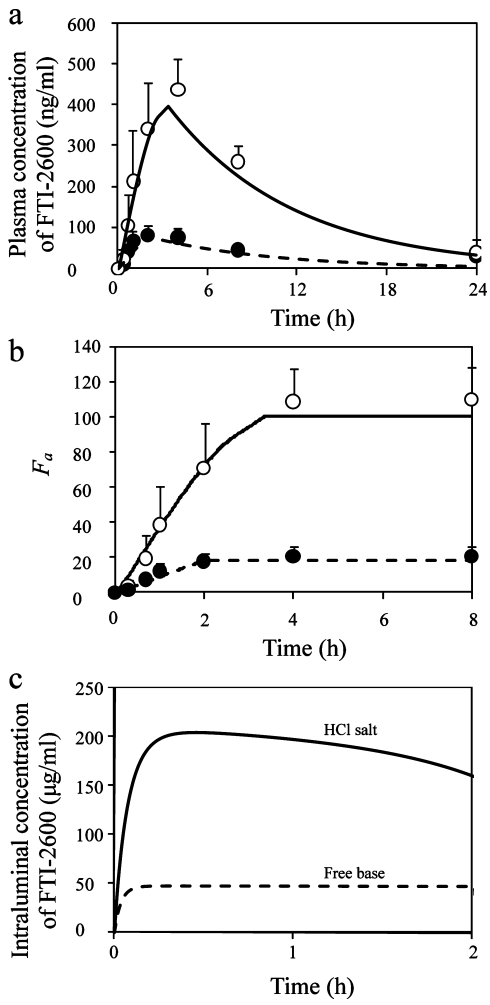

Figure 6. Comparison between the observed and simulated oral absorption of FTI-2600 crystalline free base and HCl salt. (a) The mean plasma concentration time profiles and fitting curves of the crystalline free base (● − −) and the HCl salt (○ —). A physiologically based model from eqs 1−5 was used to simulate the determined parameters listed in Table 3. (b) The mean F_a time profiles and fitting curves of the crystalline free base (● − −) and the HCl salt (○ —). Observed F_a was determined using a numerical deconvolution technique following oral administration in beagle dogs in Figure 5. By fitting the simulated profiles of F_a to those observed, the equilibrium solubility ($C_{s,vivo}$) in the small intestine in dogs after administrations of the crystalline free base and the HCl salt were determined to be 50 ± 14 and 244 ± 108 μg/mL, respectively. (c) The intraluminal drug concentration of FTI-2600 in dogs after oral administration of the crystalline free base and the HCl salt simulated using eqs 1−3 with the $C_{s,vivo}$ determined. The intraluminal concentration after the administration of crystalline free base was almost the same as the equilibrium solubility (50 μg/mL) in the small intestine, indicating that the absorption is limited by its solubility. A 4-fold increased in concentration compared to the equilibrium solubility of the crystalline free base was observed after the administration of the HCl salt.

Table 3. Parameters in Eqs 1−5 Used for the Estimation of the Time Profiles of the Plasma Concentrations, F_a and the Intraluminal Concentration in Dogs

		parameters for dissolution rate and solubility	
	PK parameters	crystalline free base	HCl salt
intestinal fluid volume V_{vivo} (mL/kg)[a]	4		
absorption rate k_a (1/min)[b]	0.023		
dissolution rate parameter z (mL/mg/min)[c]		0.59	0.33[d]
intestinal solubility $C_{s,vivo}$ (μg/mL)[e]		50	244
elimination rate k_{el} (1/min)[f]	0.002		
F_gF_h[g]	0.54		

[a] Parameter in dogs previously reported (ref 4). [b] The absorption rate in dogs for solution administration. The absorption rate was determined by deconvolution analysis. [c] z values in $FaSSIF_{dog}$. [d] Dissolution rate of the precipitant of HCl salt. [e] C_s obtained by fitting the time profiles of F_a simulated from the physiologically based model to the observed data in Figure 6b. [f] k_{el} in dogs obtained from the PK data after intravenous administration. [g] F_gF_h derived from the oral and intravenous administration of the solution.

difference in the concentration of supersaturation and the equilibrium solubility of the stable form.[44−46] Hence, the greater the degree of supersaturation, the higher the possibility for a solid-state transformation will be. For the HCl salt of FTI-2600, because the amorphous form immediately precipitated, the difference in supersaturation and the equilibrium solubility of the crystalline free base was only 4-fold. This rather small concentration difference may slow down the conversion into the stable crystalline free base and thus maintain supersaturation in the GI tract. Both the nucleation rate and the crystal growth rate are altered by a precipitation inhibitor (e.g., hydroxypropyl methylcellulose).[47] Recently, Guzman et al. reported a high potency to generate supersaturation using salts with precipitation inhibitors.[9] Celecoxib sodium or sodium propylene glycol salt crystals with excipients as precipitation inhibitors showed 1000-fold greater supersaturation in vitro and 3-fold better oral bioavailability in dogs compared to the crystalline free form. Our next challenge is to determine the factors to maintain supersaturation and design more effective formulations to improve the absorption.

Drug dissolution in the stomach and the time required for gastric emptying are critical in the oral absorption of drugs from crystalline salts because dissolution and precipitation occur in the stomach. However, in the case of the HCl salt of FTI-2600, since supersaturation was sustained in the

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simulated gastric fluid, the precipitant of the drug in the amorphous state could enter into the intestinal tract regardless of the gastric emptying time. Moreover, according to the initial absorption rate of FTI-2600 (Figure 6b), gastric emptying appeared to be rapid. Therefore, in calculating the intraluminal concentration of FTI-2600, the time element of gastric emptying could be ignored. As a result, the simulation data corresponds well to the observed data, confirming the reliability of our model of oral absorption even though it consists of a simple one-compartment of small intestine. However, if the drug concentration in the stomach is time-dependent, drug dissolution and precipitation in the stomach should be included in the model.[2,48]

In conclusion, we have successfully demonstrated the in vivo/in vitro correlation in oral drug absorption from the supersaturable salt and indicated that generating supersaturation could be a favorable strategy to improve the solubility-limited nonlinear absorption. The fact that the effect of supersaturation on oral drug absorption can be predicted from a miniscale in vitro test would promote the appropriate formulation design at the early stage of drug development leading to more success in the development of oral products of poorly water-soluble drugs.

Acknowledgment. The authors thank Ms. N. Shinagawa for her help with the in vitro dissolution tests.

Supporting Information Available: Solid state characterization and crystallographic data of the FTI-2600 HCl salt and the crystalline free base. This material is available free of charge via the Internet at http://pubs.acs.org.

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