Prediction of Oral Absorption of Low-Solubility Drugs by Using Rat Simulated Gastrointestinal Fluids: The Importance of Regional Differences in Membrane Permeability and Solubility

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ABSTRACT - Purpose. This study aimed to develop a novel approach for predicting the oral absorption of low-solubility drugs by considering regional differences in solubility and permeability within the gastrointestinal (GI) tract. **Methods.** Simulated GI fluids were prepared to reflect rat *in vivo* bile acid and phospholipid concentrations in the upper and lower small intestine. The saturated solubility and permeability of griseofulvin (GF) and albendazole (AZ), a drug with low aqueous solubility, were measured using these simulated fluids, and fraction absorbed (Fa) at time t after oral administration was calculated. **Results.** The saturated solubility of GF and AZ, a drug with low aqueous solubility, differed considerably between the simulated GI fluids. Large regional differences in drugs concentration were also observed following oral administration *in vivo*. The predicted Fa values using solubility and permeability data of the simulated GI fluid were found to correspond closely to the *in vivo* data. **Conclusion.** These results indicated the importance of evaluating regional differences in drug solubility and permeability in order to predict oral absorption of low-solubility drugs accurately. The new methodology developed in the present study could be useful for new oral drug development.

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INTRODUCTION

Prediction and assessment of human oral absorption of drug candidates at the preclinical phase is very important for the development of oral products, and there is a global research effort in this area **(1-4)**. Dissolution is the first step in oral absorption of solid drugs such as tablets and capsules. Bile acid and phospholipid concentrations in the gastrointestinal (GI) tract are important factors influencing the dissolution rate, solubility, and membrane permeability of drugs especially low soluble drugs, because of their influence on micellization **(5-7)**. Various simulated GI fluids, including bile acids and phospholipids, have been developed to predict and assess drug solubility and membrane permeability in the GI tract **(8-11)**. However, these simulated GI fluids were prepared according to the composition of the upper small intestinal fluid in humans and dogs. We have previously reported large regional differences in total bile acid and phospholipid concentrations between the upper and lower small intestine in rats **(12)**. In order to increase the accuracy of prediction

of *in vivo* oral absorption, the *in vitro* or *in situ* data related to solubility and permeability must be generated under conditions that are as close as possible to those *in vivo*. Therefore, evaluating the dissolution and solubility of drugs in the lower region of the GI tract, as well as the upper small intestine, is very important for accurate prediction of oral bioavailability. This is particularly important for poorly water-soluble drug candidates, because dissolution and solubility of these compounds are strongly influenced by regional differences in bile acid and phospholipid concentrations. Fotaki et al. and Jantratid et al. used media simulating human proximal colon and ileum fluids to evaluate the dissolution of drugs in these regions **(13, 14)**. Although these media were prepared to match the osmolality, buffering capacity, and ionic strength of actual GI fluids, the bile acids and phospholipids concentration might not be adjusted to *in vivo*,

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because it might be assumed that bile acids and phospholipids were completely absorbed or degraded in these regions.

When intestinal drug concentration reaches and sustains its saturated solubility level, the amount absorbed can be expressed as a product of this drug saturated solubility, the intestinal membrane permeability, the surface area of the intestine, and the drug residence time **(15, 16)**. The amount of drug absorbed estimated in this way is known as the maximum absorbable dose (MAD), because the drug is assumed to maintain saturated drug solubility throughout its transit through the GI tract. When the dose-to-luminal volume ratio is higher than the solubility of the drug (dose number) and the dissolution rate is greater than the absorption rate across the GI membrane, drug absorption is limited by solubility and not by the dissolution rate **(17, 18)**. This solubility-limited absorption is often observed when poorly water-soluble drugs are administered orally. The equation to estimate MAD is therefore considered very useful for the evaluation and/or prediction of the oral absorption of low-solubility drugs.

In this study, fasted state simulated rat intestinal fluids were formulated based on information about total bile acid and phospholipid concentrations in the upper and lower small intestines **(12)**. Using these, a novel method for predicting oral absorption of a drug with low aqueous solubility was developed. This method used the MAD equation and took into account regional differences in drug solubility and intestinal membrane permeability within the GI tract.

MATERIALS AND METHODS

Materials
Albendazole (AZ) was purchased from Tokyo Kasei Kogyo Co., Ltd. (Tokyo, Japan). Griseofulvin (GF), albendazole sulfoxide (AZSO; an active metabolite of AZ), egg-lecithin and sodium taurocholate were purchased from WAKO Pure Chemical Industries, Ltd. (Osaka, Japan). All other reagents were analytical-grade commercial products.

Preparation of fasted state simulated GI fluids
The simulated GI fluid compositions are summarized in Table 1. Isotonic phosphate buffers (pH 7.0) were prepared by mixing 2.54% $NaH_2PO_4 \cdot 2H_2O$ and 4.41% $Na_2HPO_4 \cdot 12H_2O$. Then, distilled water was added to the pH 7.0 phosphate buffer solution in the ratio of 4:16 or 6.8:13.2. Sodium taurocholate and egg-lecithin were dissolved in phosphate buffer diluted with water at 4:16 or 6.8:13.2 to prepare simulated upper GI tract fluid ($FaSSIF_{rat, upper}$) or lower GI tract fluid ($FaSSIF_{rat, lower}$), respectively. The osmotic pressures of $FaSSIF_{rat, upper}$ and $FaSSIF_{rat, lower}$ were 280–290 mOsmol/kg (OSMOMAT, 030-3P, GONOTEC GmbH, Germany).

Prediction of fraction absorbed (Fa) at time t
The amount of drug absorbed from the GI tract at time t under solubility-limited conditions was estimated using the following equation **(15, 16)**:

Amount absorbed at time t

$$(Xa_t) = P \times Cs \times S \times t \qquad (1)$$

P, Cs, S, and t are the intestinal membrane permeability, the saturated drug solubility in intestinal fluid, the intestinal surface area, and the drug duration time in the GI tract, respectively. In order to consider the regional differences in each parameter, the GI tract was divided into two segments (the upper small intestine and lower small

Table 1. The composition of $FaSSIF_{rat, upper}$ and $FaSSIF_{rat, lower}$

	Sodium taurocholate (mM)	Egg-lecithin (mM)	pH
$FaSSIF_{rat, upper}$	50	3.7	7.0
$FaSSIF_{rat, lower}$	100	0.1	7.0

The bile acid and phospholipids concentrations and pH were referred to previous report (12).

intestine), and the drug absorption from each segment at time t was calculated using the relevant P, Cs, S, and t of that GI segment. The total amount absorbed at time t from the whole small intestine was estimated using the sum of the absorbed amounts at time t from each segment, as shown below.

Total amount absorbed at time t
$$(Xa_{total, t}) = Xa_{upper, t} + Xa_{lower, t} = P_{upper} \times Cs_{upper} \times S_{upper} \times t_{upper} + P_{lower} \times Cs_{lower} \times S_{lower} \times t_{lower} \qquad (2)$$

Where $Xa_{upper, t}$, P_{upper}, Cs_{upper}, S_{upper}, t_{upper} and $Xa_{lower, t}$, P_{lower}, Cs_{lower}, S_{lower}, and t_{lower} are the amount of drug absorbed at time t, the GI membrane permeability, the saturated drug solubility, the GI membrane surface area, and the duration time of saturated drug solubility in the upper small intestine and lower small intestine, respectively. The predicted Fa value of a drug at time t ($Fa_{predicted, t}$) was then estimated by dividing $Xa_{total, t}$ by the dose.

$$Fa_{predicted, t} = Xa_{total, t}/Dose \qquad (3)$$

Saturated solubility measurement of GF and AZ in simulated GI fluids
Saturated GF and AZ solubility in $FaSSIF_{rat, upper}$ and $FaSSIF_{rat, lower}$ was measured to provide values for Cs_{upper} and $Cs_{lower,}$ respectively. Excess GF or AZ was suspended in $FaSSIF_{rat, upper}$ and $FaSSIF_{rat, lower}$, and vortexed. Each sample was then shaken in an incubator at 37°C for 24 h. The resulting suspensions were filtered through 0.45-µm cellulose membranes before analyzing GF and AZ in the supernatant by high-performance liquid chromatography (HPLC).

Measurement of the rat small intestine length
All animal studies were conducted in accordance with the Guidelines for the Care and Use of Laboratory Animals of the Committee for Animal Experiments of Hiroshima International University. The whole small intestine (from the pyloric sphincter to the ileocecal valve) was removed. The length was directly measured using a ruler. This length was used to calculate the surface area in each GI region (S_{upper} and S_{lower}).

In situ measurement of the apparent permeability of GF and AZ
The apparent permeability of GF and AZ in the upper and lower small intestine (P_{upper} and P_{lower}) was measured using the *in situ* closed loop method. GF and AZ was dissolved in $FaSSIF_{rat, upper}$ and $FaSSIF_{rat, lower}$ at a concentration of 50 and 100 µg/mL for GF and 8 and 16 µg/mL for AZ, respectively. Each drug solution (1 mL/10 cm) was injected into the corresponding GI segment, and both ends were ligated to make intestinal loops. After a period of time, the luminal solution was collected. The amount absorbed was calculated by subtracting the amount remaining from the original amount in the loop. The apparent GF and AZ permeability in each GI segment (P_{upper} and P_{lower}) was evaluated using the following equation **(19)**.

$$P = kaV/2\pi RL \qquad (4)$$

Where ka is the drug absorption rate constant estimated from the absorbed amount during the defined period, assuming that drug absorption follows first-order kinetics; V is the volume of drug solution injected into each loop; and R is the radius of the GI tract (0.18 cm for the upper and lower small intestine **(20)**. L is the length of GI tract used in this experiment.

In vivo measurement of luminal and plasma drug concentrations
GF and AZ powders were suspended in 0.5% methylcellulose 50 solutions at 25 and 3.5 mg/mL, respectively. After oral administration of 1 mL drug suspension to fasted rats (210–240 g), they were killed at the time points indicated. The abdomen was then opened immediately to collect samples of luminal fluid from the stomach, upper small intestine (from a site 5 to 30 cm distal to the stomach), and lower small intestine (from a site 5 to 30 cm proximal to the cecum), by using a micropipette. To sample colonic fluid, luminal contents of the rectum and colon were collected using a microspatula due to the very small amount of colonic fluid. These samples were then immediately filtered through 0.65-µm hydrophilic polyvinylidene fluoride (PVDF) centrifugal filter units (Ultrafree®, Millipore Corporation, USA), and the filtrates were regarded as a luminal fluid. The filtrates were weighed and the volumes were calculated by assuming a relative density of 1. The filtrates were then diluted with 10% methanol for GF and 25% dimethyl sulfoxide (DMSO) solution for AZ. Adsorption of AZ to the microtube surface because of high hydrophobicity can be avoided by

adding DMSO.

Blood samples (1 mL) were taken from the jugular vein at the same time as the GI fluid sampling. Portal blood samples were also taken in a group of rats administered GF suspension. Plasma was obtained by centrifugation and de-proteinized by acetonitrile precipitation. After centrifugation, the resulting supernatant was evaporated prior to resuspension in the HPLC mobile phase for GF and 20% DMSO solution for AZSO and determination of GF and AZSO concentration by using HPLC. After intestinal absorption, almost 100% of AZ is rapidly oxidized into its pharmacologically active metabolite, AZSO, in the mucosal cells and the liver and subsequently AZSO converted into the inactive metabolite albendazole sulfone **(21, 22)**. Hence, the AZSO concentration was quantified for the evaluation of oral AZ absorption.

Measurement of the saturated solubility of AZ in the upper and lower intestinal fluid samples from each segment of the rat GI tract

Upper and lower intestinal fluids were taken from rat GI tract. Then, excess AZ was suspended in each luminal fluid sample and was vortexed sufficiently. Then, each sample was shaken in an incubator at 37°C for 3 hr. The suspension was filtered through a 0.65 μm hydrophilic polyvinylidene fluoride membrane (Millipore Corporation, USA). The supernatant was analyzed by HPLC.

In vivo oral and intravenous (i.v.) administration

GF powder was dissolved in 60% polyethyleneglycol 400 (PEG400) solution at a concentration of 1 or 0.5 mg/mL. Then, 0.5 mL of 1 mg/mL GF solution was administered i.v. to fasted rats (210–240 g) via the jugular vein (0.5 mg/body) and 1 mL of 0.5 mg/mL GF solution was administered orally (0.5 mg/body). AZSO powder was dissolved 40% PEG400 and 10% DMSO solution at a concentration of 0.5 mg/mL, and then the 0.4 mL (0.2 mg/body) was injected via the jugular vein. Femoral artery blood samples were obtained periodically via a cannula. Plasma was prepared by centrifugation and de-proteinized by acetonitrile precipitation. After centrifugation, the resulting supernatant was evaporated and then resuspended in the HPLC mobile phase and 20% DMSO solution for GF and AZSO, respectively. The drug concentration in each sample was then analyzed using HPLC.

Measurement of portal plasma concentration after oral administration of GF solution

GF solution (0.5 mg/mL, 1 mL) with 60% PEG400 was administered orally to fasted rats. Then, the rats were killed periodically, and the abdomen was opened. Blood samples were taken from the portal vein and centrifuged to obtain plasma samples. After de-proteinization by acetonitrile precipitation, the resulting supernatant was evaporated prior to resuspension in the HPLC mobile phase and determination of GF concentration by using HPLC.

Calculation of the *in vivo* Fa

In the case of GF, $BA_{suspension}$ at time t ($BA_{suspension, t}$) was estimated from the time-course of oral bioavailability (BA) which was calculated by a deconvolution method using the plasma GF concentrations after oral administration of 25 mg GF suspension and after i.v. administration of GF solution. The fraction GF absorbed after oral administration of suspension at time t ($Fa_{suspension, t}$) was calculated as shown below.

$$BA_{solution} = AUC_{0-\infty, solution}/AUC_{0-\infty, i.v.} \times Dose_{i.v.}/Dose_{solution} \times 100 \quad (5)$$
$$= Fa_{solution} \times Fg_{solution} \times Fh_{solution} \times 100 \quad (6)$$
$$= Fg_{solution} \times Fh_{solution} \times 100 \quad (7)$$

$$Fa_{suspention, t} = BA_{suspension, t}/BA_{solution} \quad (8)$$
$$= (Fa_{suspension, t} \times Fg_{suspension} \times Fh_{suspension})/(Fg_{solution} \times Fh_{solution})$$

$AUC_{0-\infty, solution}$ and $AUC_{0-\infty, i.v.}$ refer to the areas under the curve of the plasma GF concentration time-course from 0 h to infinite time after oral or i.v. administration of GF solutions, respectively. The AUC from 0 h to infinite time ($AUC_{0-\infty}$) was calculated by extrapolation using several points of terminal plasma drug concentrations in the time-profiles.

The $Fa_{solution}$ (fraction absorbed after oral administration of GF solution) was regarded as 1, because the drug was administered as a solution and could therefore be completely absorbed from the GI tract because of the high permeability. If linear drug metabolism kinetics in the GI mucosa (Fg) and the liver (Fh) were assumed for both administrations of solution ($Fg_{solution}$ and $Fh_{solution}$, respectively) and suspension ($Fg_{suspension}$ and $Fh_{suspension}$, respectively), the relative BA of $BA_{suspension, t}$ to $BA_{solution}$ gave the $Fa_{suspension, t}$.

However, if non-linearity was observed in

$Fg_{suspension}$ and $Fh_{suspension}$ in the administration of the GF suspension, due to the much higher dose than the solution, the $Fa_{suspension, t}$ becomes the apparent value multiplied by the increasing rate of $(Fg_{suspension} \times Fh_{suspension})/(Fg_{solution} \times Fh_{solution})$. Therefore, in order to correct the apparent value to the true $Fa_{suspension, t}$ value, $Fh_{suspension}/Fh_{solution}$ was calculated as follows.

$$Fa \times Fg = Q_{pv} \times Rb \times (AUC_{pv} - AUC_{sys})/Dose \quad (9)$$

Where Q_{pv}, Rb, AUC_{pv}, and AUC_{sys} are the portal blood flow, the blood/plasma concentration ratio, AUC in the portal vein, and AUC in the systemic circulation, respectively **(23, 24)**.

$Fh_{suspension}$ and $Fh_{solution}$ can be calculated by equations (10) and (11).

$$Fh_{suspension} = BA_{suspension}/(Fa_{suspension} \times Fg_{suspension})$$
$$= BA_{suspension}/(Q_{pv} \times Rb \times (AUC_{0-\infty, suspension, pv} - AUC_{0-\infty, suspension, sys})/Dose) \quad (10)$$

$$Fh_{solution} = BA_{solution}/(Fa_{solution} \times Fg_{solution})$$
$$= BA_{solution}/(Q_{pv} \times Rb \times (AUC_{0-\infty, solution, pv} - AUC_{0-\infty, solution, sys})/Dose) \quad (11)$$

Where $AUC_{0-\infty, suspension, pv}$, $AUC_{0-\infty, suspension, sys}$, $AUC_{0-\infty, solution, pv}$ and $AUC_{0-\infty, solution, sys}$ are the AUC at time 0 h–infinity in the portal vein and systemic circulation after oral administration of GF suspension and solution, respectively.

$Fh_{suspension}/Fh_{solution}$ were estimated using equations (10) and (11).

$$Fh_{suspension}/Fh_{solution}$$
$$= (BA_{suspension}/((AUC_{0-\infty, suspension, pv} - AUC_{0-\infty, suspension, sys})/Dose))/$$
$$(BA_{solution}/((AUC_{0-\infty, solution, pv} - AUC_{0-\infty, solution, sys})/Dose)) \quad (12)$$

Q_{pv} and Rb were assumed to be the same value in the administrations of both GF suspension and solution.
$Fa_{suspension, t}$ was corrected by dividing by the $Fh_{suspension}/Fh_{solution}$.

$$Fa_{suspension, t, cor.} = Fa_{suspension, t}/(Fh_{suspension}/Fh_{solution}) \quad (13)$$

In the case of AZ, *in vivo* Fa of AZ was estimated in the almost same way depicted in equations (5) and (8). In the present study, BA of AZ was defined as the rate of AZSO amount reaching systemic circulation to oral AZ dosage, because the first-pass effect of AZ into AZSO is almost 100% **(21, 22)**. Deconvolution using the AZSO plasma concentration after oral administration of 3.5 mg of AZ as suspension and i.v. administration of 0.2 mg of AZSO gives BA of AZ at each time point ($BA_{suspension, t}$). $BA_{solution}$ calculated using $AUC_{0-\infty, solution}$ after oral administration of AZ as solution and $AUC_{0-\infty, i.v.}$ after i.v. administration of AZSO provides $Fg \times Fh$ of AZSO in the first pass, because AZ administered as solution is completely absorbed from the GI tract due to the high permeability as with GF. If AZSO is not metabolized in the first pass at all, the $AUC_{0-\infty, solution}$ become equal to $AUC_{0-\infty, i.v}$ when the doses in the both administrations are same. Hence, the relative BA of $BA_{suspension, t}$ to $BA_{solution}$ gave the $Fa_{suspension, t}$ of AZ. These calculations were conducted after converting the unit of dose (mg) and plasma concentration ($\mu g/mL$) to molar unit.

HPLC analysis

HPLC with a pump (LC-20AD, Shimadzu Corporation, Kyoto, Japan) and a UV detector (SPD-20A, Shimadzu Corporation, Kyoto, Japan) was used for quantification of GF, AZ and AZSO. Column oven was set at 40 °C . An analytical column (YMC-Pack Pro C18; 150 × 6.0 mm I.D.; YMC Co., Ltd. Japan) was used for GF and AZ. The mobile phases consisted of 50 mM phosphate buffer (pH, 6.0 for GF and pH, 2.5 for AZ) and acetonitrile in a ratio of 11:9 and 6:4 (v/v) for GF and AZ, respectively. The concentration of AZSO in the plasma samples was determined using a Zorbax Eclipse XDB-C18 column (2.1 × 50 mm, I.D., 5 μm, Agilent Technologies). The mobile phases consisted of 50 mM phosphate buffer (pH, 4.0) and acetonitrile in a ratio of 9:1. GF, AZ, and AZSO were detected at 293, 310, and 292 nm, respectively.

RESULTS

Solubility of GF and AZ in FaSSIF$_{rat, upper}$ and FaSSIF$_{rat, lower}$

FaSSIF$_{rat, upper}$ and FaSSIF$_{rat, lower}$, were prepared based on the total bile acid, phospholipid concentrations and pH found in the upper and lower small intestine (Table 1) **(12)**. The saturated solubility of GF and AZ in each simulated GI fluid (Cs_{upper} and Cs_{lower}) is shown in Fig. 1A and B.

These values were 104.6 ± 6.5, and 230.0 ± 13.3 µg/mL for GF and 14.9 ± 0.31, and 35.0 ± 0.77 µg/mL for AZ in $FaSSIF_{rat, upper}$ and $FaSSIF_{rat, lower}$, respectively. It is considered that the difference in the solubility values in $FaSSIF_{rat, upper}$ and $FaSSIF_{rat, lower}$ depends on the taurocholic acid concentration in each simulated GI fluid. The saturated solubility of GF and AZ in $FaSSIF_{rat, upper}$ and $FaSSIF_{rat, lower}$ were almost the same values as those in *in vivo* rat upper and lower jejunal fluids (122.4 ± 42.0 and 213.0 ± 85.1 µg/mL for GF **(12)**, and 14.0 ± 6.92 and 30.8 ± 13.3 µg/mL for AZ, respectively). Therefore, the developed simulated GI fluids correlated well with *in vivo* solubility behavior.

Apparent surface area and permeability of each GI tract

The length of the small intestine was 72.2 ± 6.1 cm. In the present study, the lengths of the upper and lower small intestine were defined as 36.1 cm by dividing in the middle of the small intestine. The apparent surface area of each GI segment was calculated as 40.8 cm^2 for the upper and lower small intestine (S_{upper} and S_{lower}) by using a radius of 0.18 cm (Table 2) **(20)**.

The drug permeability in each GI region estimated by using the corresponding simulated GI fluid (P_{upper} and P_{lower}) was 3.4 ± 0.35 and $3.2 \pm 0.32 \times 10^{-5}$ cm/s for GF and 3.2 ± 0.18 and $4.0 \pm 1.0 \times 10^{-5}$ cm/s for AZ, respectively.

Estimation of the duration of saturated GF and AZ solubility in each GI tract region

Luminal GF and AZ concentrations were measured to quantify the duration time of the saturated solubility of GF and AZ in the upper small intestine (t_{upper}) and lower small intestine (t_{lower}) (Figs. 2 and 3). The solid and dotted lines in Figs. 2 and 3 show the saturated solubility and the standard deviation in *in vivo* upper and lower small intestinal fluids. The GF concentration in the stomach 0.5 h after oral administration of 25 mg GF suspension was approximately 20.5 µg/mL, and this concentration decreased gradually with time. The luminal concentration in the upper and lower small intestine showed the saturated solubility for 2.5 and 3 h, respectively. Therefore, the duration time of the GF saturated solubility was 0–2.5 h for the upper small intestine, and 0–3.0 h for the lower small intestine. In AZ, the AZ concentration in the stomach reached about 100 µg/mL at highest. The concentration was much higher than those in the upper or lower small intestine, because AZ shows basic properties **(25)**. However, no supersaturation phenomenon of AZ in upper small intestine was observed due to rapid precipitation. The luminal AZ concentration in the upper and lower small intestine showed the saturated solubility for 1.0 and 2.5 h, respectively. Therefore, the duration time of the AZ saturated solubility was decided as 0–1.0 h for the upper small intestine, and 0–2.5 h for the lower small intestine. The AZ concentration in upper small intestine increased again at 4h (Fig. 3).
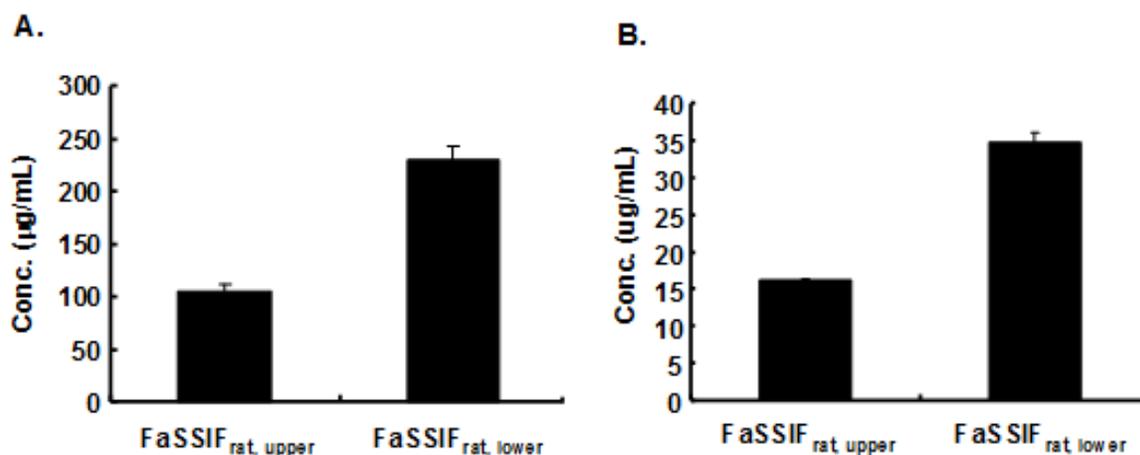
Figure 1. The GF (A) and AZ (B) solubility in FaSSIF $_{rat, upper}$ and FaSSIF rat, lower* The results are expressed as the mean ± SD (N=64).

Table 2. Apparent surface area (N=7) and permeability of GF (N=5) and AZ (N=4) in each GI segment

GI segments	Surface area (cm^2)	Permeability ± SD ($\times10^{-5}$ cm/sec)	
		GF	AZ
Upper small intestine	40.8	3.4 ± 0.35	3.2 ± 0.18
Lower small intestine	40.8	3.2 ± 0.32	4.0 ± 1.0
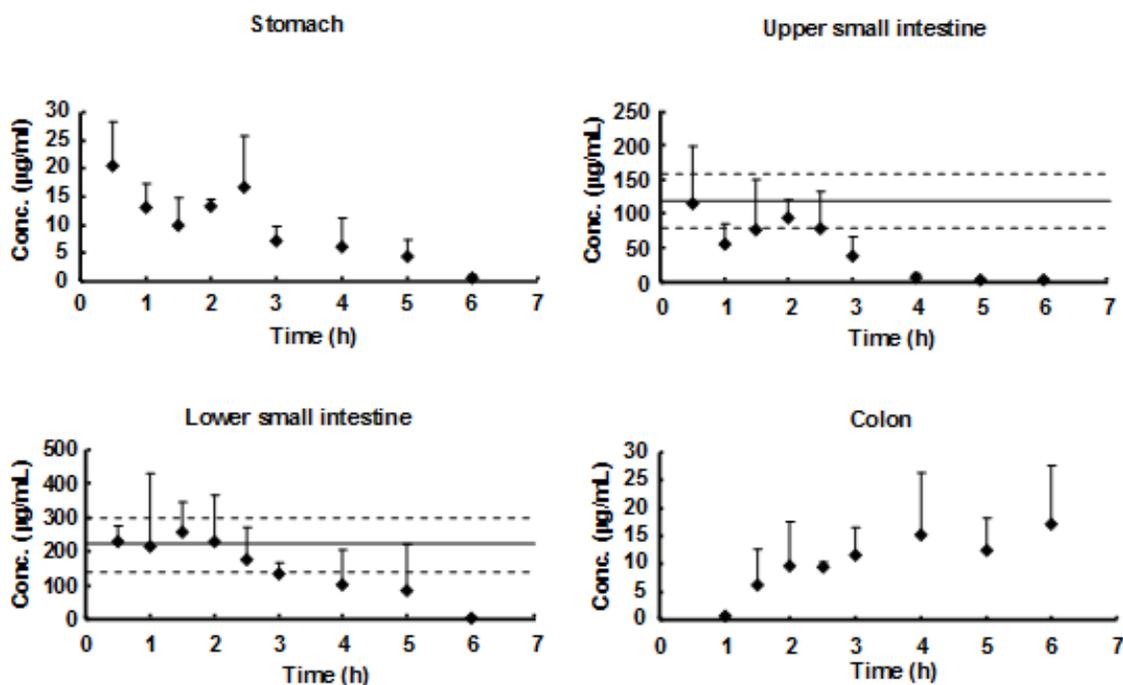
Figure 2. Luminal GF concentrations after oral administration. The results are expressed as the mean ± SD (N=4).

This is because that one of the four upper intestinal samples at 4 h obtained from rats showed much higher AZ concentration, and it might be attributed to large individual difference in intestinal transit of drug particles. In colon, the GF and AZ concentrations were much lower than those in upper and lower small intestine. This is due to much lower concentration of total bile acid and phospholipids concentration in colon. The total bile acid and phospholipids concentrations were about 50 and 3.7 mM in upper small intestine and 100 and 0.1 mM in lower small intestine, respectively **(12)**. These concentrations in colon was determined by same methods reported in our previous work **(12)**, and the total bile acid concentration was 9.2 ± 4.0 mM, and no phospholipids were detected in colonic fluid.

Plasma GF and AZSO concentration and the PK parameters

The plasma concentration-time profiles after oral and i.v. administration of GF are shown in Fig. 4A and B, and the pharmacokinetics parameters are summarized in Table 3. The AUC values after oral administration of 25 mg GF suspension and 0.5 mg GF solution were 7.53 ($AUC_{0-\infty, \text{ suspension, sys}}$) and 0.421 ($AUC_{0-\infty, \text{ solution, sys}}$) µg × h/mL, respectively, in the systemic plasma and 14.2 ($AUC_{0-\infty, \text{ suspension, pv}}$) and 0.875 ($AUC_{0-\infty, \text{ solution, pv}}$) µg × h/mL, respectively, in the portal plasma. No GF was determined in the lower small intestinal fluid after oral administration of 0.5 mg of GF solution, indicating GF was completely absorbed (data not shown), and therefore Fa_{solution} could be assumed to be 1. The AUC was 1.44µg × h/mL after i.v. administration of GF solution ($AUC_{0-\infty, \text{ i.v.}}$).

$BA_{suspension}$ and $BA_{solution}$ values, calculated by comparing $AUC_{0-\infty, suspension, sys}$ and $AUC_{0-\infty, solution, sys}$ with $AUC_{0-\infty, i.v.}$, were 10.6% and 29.8%, respectively. The non-linearity in GF metabolism in the liver after oral administration of GF suspension was observed, because the $Fh_{suspension}/Fh_{solution}$ value was about 1.22. In AZ (Fig 5 and Table 3), the AUC values estimated from AZSO concentration-time profiles after oral administration of 3.5 mg AZ suspension and i.v. administration of 0.2 mg AZSO solution were 12.8 ($AUC_{0-\infty, suspension, sys}$) and 3.01 ($AUC_{0-\infty, i.v.}$) µg × h/mL, respectively, and $BA_{suspension}$ of AZ was 24.2%. The AUC (3.72 µg × h/mL) calculated from AZSO concentration-time profile after oral administration of 0.275 mg AZ solution ($AUC_{0-\infty, solution, sys}$) was reported in our previous study **(26)**, and used for estimation of $BA_{solution}$ of AZ. The value was 90%. We measured the levels of intact AZ after oral administration of AZ by using HPLC at the same time as AZSO quantification (data not shown). The HPLC conditions for AZ have been reported previously **(27)**. Although a very low concentration of AZ was detected (C_{max} values were 0.06 and 0.04 µg/mL after oral administration of 3.5 mg and 0.275 mg of AZ as suspension and solution, respectively.), it is considered to be negligible level and therefore, it was indicating almost 100% of AZ absorbed across the GI tract was metabolized into AZSO.

Fa values

In vivo and predicted Fa values are shown in Table 4. The equation for MAD used for estimation of predicted Fa in this study can estimate absorbed drug amount only under solubility-limited absorption (In other words, under condition that luminal drug concentration sustains the saturated solubility) and the GF and AZ concentration in the lower small intestine after oral administration of these suspensions sustained the saturated solubility until 3 h and 2.5 h, respectively. Therefore, the predicted Fa values at time 3 h ($Fa_{predicted, 3h}$) for GF and at time 2.5 h ($Fa_{predicted, 2.5h}$) for AZ were calculated using the apparent permeability and saturated solubility of GF and AZ, estimated using two different types of simulated GI fluid, the surface area, and the duration of saturated GF and AZ in each GI segment (GF and AZ dose = 25 and 3.5 mg, respectively). In addition, Fa values at time t were also predicted only using permeability and saturated solubility data of GF and AZ estimated using $FaSSIF_{rat, upper}$, instead of that estimated using $FaSSIF_{rat, lower}$ ($Fa_{predicted, upper, t}$), to evaluate the importance of consideration of regional difference in the drug dissolution and permeation for prediction of oral absorption. The predicted Fa values at 3 h for GF ($Fa_{predicted, 3h}$ and $Fa_{predicted, upper, 3h}$) and at 2.5 h for AZ ($Fa_{predicted, 2.5h}$ and $Fa_{predicted, upper, 2.5h}$) were calculated by adopting t_{upper} (2.5 h or 1 h) and t_{lower} (3 h or 2.5h), respectively. To compare with predicted Fa values of GF and AZ, the *in vivo* Fa value at 3 h ($Fa_{suspension, 3h, cor.}$) for GF and at 2.5 h ($Fa_{suspension, 2.5h}$) for AZ were calculated using equations (5) and (8) and/or (13).
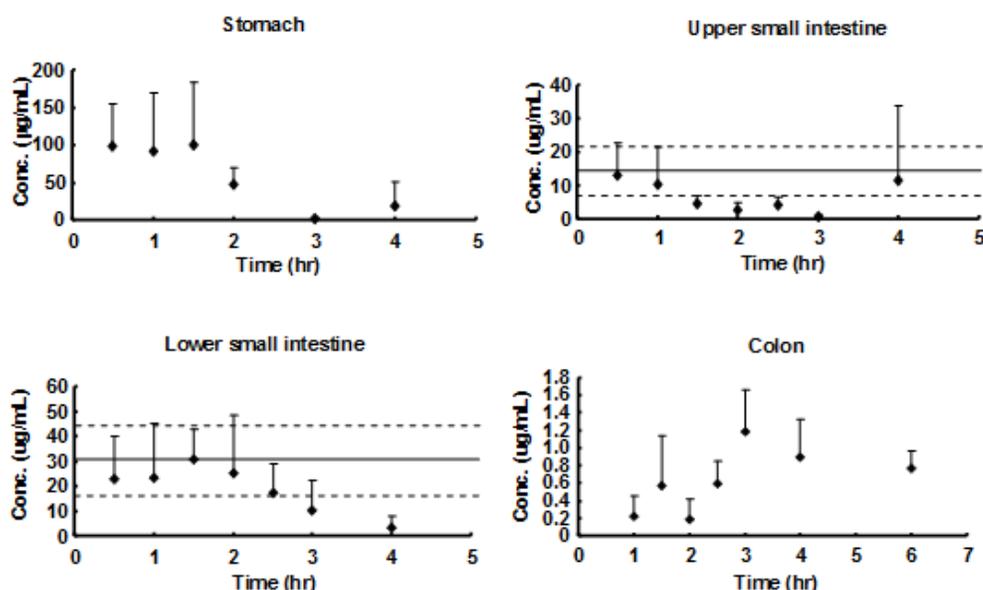
Figure 3. Luminal AZ concentrations after oral administration. The results are expressed as the mean ± SD (N=4).
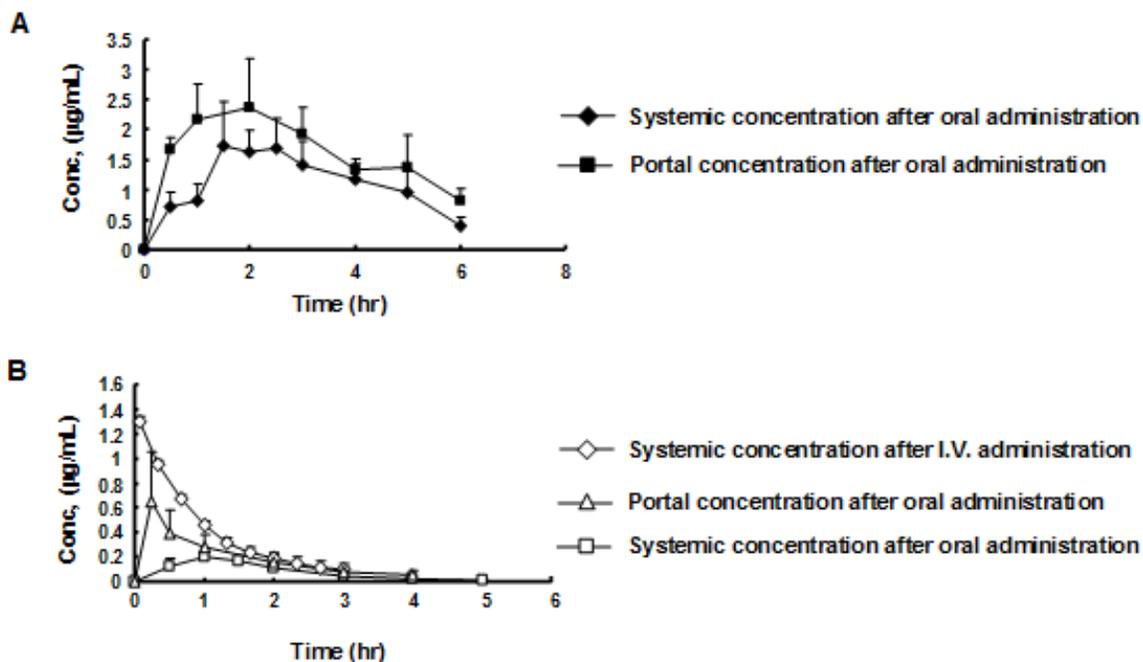
Figure 4. Systemic and portal plasma GF concentration-time courses after oral and i.v. administration. A; suspension (25 mg/body, N=4), B; solution (0.5 mg/body, N=5-6). The results are expressed as the mean ± SD.
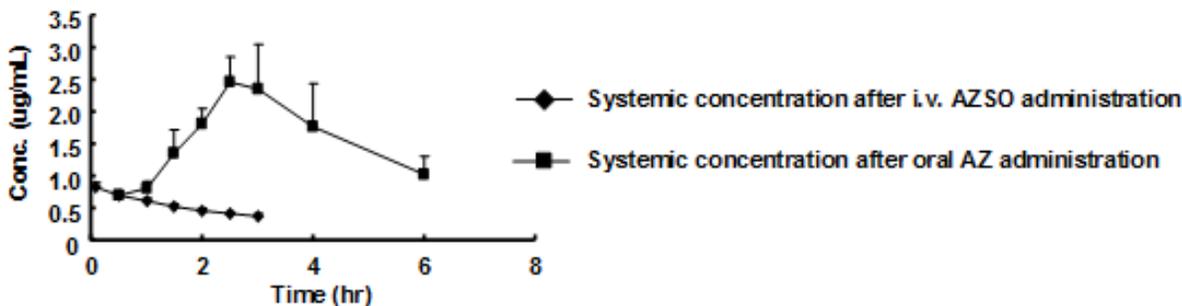
Figure 5. Plasma AZSO concentration-time courses after oral AZ administration as suspension (3.5mg) and i.v. AZSO administration (0.2mg). The results are expressed as the mean ± SD (N=4).

The $Fa_{predicted,\ 3h}$ of GF was 17.6%. This was very similar to the *in vivo* Fa value at 3 h ($Fa_{suspension,\ 3h,\ cor.}$, 20.8%). In contrast, the predicted Fa value at 3 h estimated using $FaSSIF_{rat,\ upper}$ ($Fa_{predicted,\ upper,\ 3h}$) alone was 11.5%, only about half the *in vivo* Fa value ($Fa_{suspension,\ 3h,\ cor.}$). In AZ, the $Fa_{predicted,\ 2.5h}$ (24.5%) was comparable with the $Fa_{suspension,\ 2.5h}$ (25.6%) as well as GF, and the $Fa_{predicted,\ upper,\ 2.5h}$ (11.1%) considerably underestimated the *in vivo* Fa of AZ at 2.5h. These data demonstrated that

consideration of regional differences in GF dissolution and permeation using simulated GI fluid tailored to each part of the GI tract gave better prediction accuracy for *in vivo* Fa than predictions using simulated upper GI tract fluid only. The predicted Fa values in the upper and lower small intestine were 5.2 and 12.4% in GF, and 3.2 and 21.3% in AZ, respectively, in $Fa_{predicted,\ 3h}$, and 5.2 and 6.3% in GF, and 3.2 and 7.9% in AZ, respectively, in $Fa_{predicted,\ upper,\ 3h}$.

Table 3. Phamacokinetic parameters

Parameters	GF	AZ or AZSO
$AUC_{0-\infty, \text{suspension, sys}}$	7.53 µg×h/mL	12.8 µg×h/mL[a]
$AUC_{0-\infty, \text{solution, sys}}$	0.421±0.093 µg×h/mL	3.72±0.62 µg×h/mL[b]
$AUC_{0-\infty, \text{i.v.}}$	1.44±0.21 µg×h/mL	3.01±0.48 µg×h/mL[a]
$AUC_{0-\infty, \text{suspension, pv}}$	14.2 µg×h/mL	—
$AUC_{0-\infty, \text{solution, pv}}$	0.875 µg×h/mL	—
$BA_{\text{suspension}}$	10.6%	24.2%[c]
BA_{solution}	29.8%	90%[c]
$Fh_{\text{suspension}}/Fh_{\text{solution}}$	1.22	—

[a]The AUC calculated from AZSO concentration-time profile after oral AZ administration as suspension ($AUC_{0-\infty, \text{suspension, sys}}$) and i.v. AZSO administration ($AUC_{0-\infty, \text{i.v.}}$).

[b]The AUC calculated from AZSO concentration-time profile after oral administration of 0.275 mg of AZ as solution (26).

[c]Calculated based on the AUCs from plasma AZSO concentration after iv administration of AZSO and oral administration of AZ as suspension or solution. In the present study, in the case of AZ, the BA was defined as the rate of AZSO amount reaching systemic circulation to oral dosage of AZ.

Table 4. *In vivo* and predicted Fa values in each GI segment

		Upper small intestine	Lower small intestine	Total Fa
GF	*In vivo* $Fa_{\text{suspension, 3h, cor.}}$			20.8%
	$Fa_{\text{predicted, 3h}}$ and the composition in each GI segment	5.2%	12.4%	17.6%
	$Fa_{\text{predicted, upper, 3h}}$ and the composition in each GI segment	5.2%	6.3%	11.5%
AZ	*In vivo* $Fa_{\text{suspension, 2.5h}}$			25.6%
	$Fa_{\text{predicted, 2.5h}}$ and the composition in each GI segment	3.2%	21.3%	24.5%
	$Fa_{\text{predicted, upper, 2.5h}}$ and the composition in each GI segment	3.2%	7.9%	11.1%

DISCUSSION

Fasted state simulated GI fluids reflecting the properties of the upper and lower small intestine were developed (FaSSIF$_{rat, upper}$, and FaSSIF$_{rat, lower}$) in order to predict the fraction of GF and AZ absorbed (Fa) *in vivo*. The predicted Fa value generated using these simulated GI fluids was compared with the *in vivo* Fa, and the utility of this method for prediction of *in vivo* Fa value was evaluated.

Masaoka et al. evaluated apparent intestinal permeability of GF in micelle-free solution by *in situ* single pass perfusion method, and the value in jejunum (0.909×10^{-4} cm/sec) were a little lower than those in ileum and colon (1.243 and 1.296 $\times$ 10^{-4} cm/sec, respectively) **(19)**. This is because that GF permeation across the GI membrane is limited mainly by unstirred water layer (UWL) **(28)**, and the thickness is thicker in jejunum than those in ileum and colon **(19)**. In the present study, the GF permeability estimated in FaSSIF$_{rat, upper}$ and FaSSIF$_{rat, lower}$ showed much lower values in each GI segment compared to those estimated in micelle-free solution, and the regional difference in the permeability disappeared (Table 2). It is generally considered that only the free drug concentration is available for transport across the membrane in the presence of mixed micelles **(29, 30)**. Therefore, it is considered that the presence of mixed micelles in the simulated GI fluids decreased the free fraction of GF on the intestinal membrane surface, inducing the reduction of the apparent permeability in each GI segment.

However, assuming that only free drug is available for transport across the membrane, the lower GF permeability would be expected for FaSSIF$_{rat, lower}$ because of the high sodium taurocholate concentration and the low free GF fraction. However, GF permeability estimated using the simulated GI fluids was similar in each GI tract region, and regional differences in GI membrane permeability were not observed (Table 2). Amidon et al. reported that micelles assisted the transport of solubilized drug across the aqueous diffusion layer to the surface of the intestinal membrane, leading to a reduction in the resistance of the UWL to lipophilic drug absorption **(31, 32)**. In addition, Yano et al. recently reported absorption of lipophilic drugs by direct partitioning into the intestinal membrane from the micellar phase, although the permeability was substantially lesser

than that of free drug **(33)**. Taken together, this might be because the reduction in apparent GF permeability due to the reduction in the free fraction was partially canceled out by reduced UWL resistance and direct GF permeation from the micellar phase. Therefore, the ratio of free to micellar drug concentrations is very important for estimation of *in vivo* intestinal drug permeability. The use of newly developed simulated GI fluids by taking regional differences into account can help improve estimation of drug membrane permeation. When intestinal permeability of a drug estimated in solution without micelles or of different micelle concentration from *in vivo* is used for prediction of oral absorption, the accuracy might be reduced. There are some reports to attempt the prediction of oral GF absorption in rats. Although the permeability of GF in micelle-free solution was estimated by *in situ* loop method and the data was used for the prediction in these report, the plasma GF concentration-time profiles has been well predicted. This is because that biorelevant media including much lower concentration of bile acids than the actual value was used to estimate the saturated GF solubility and the dissolution rate constant, and therefore it was considered that the overestimated apparent permeability of GF in micelle-free solution compensated underestimated the solubility or dissolution rate constant. In AZ, the permeability in lower small intestine was 1.25 times higher than that in upper small intestine (Table 2), although it was considered the free AZ fraction in FaSSIF$_{rat, lower}$ was lower than that in FaSSIF$_{rat, upper}$. The solubility of AZ in water is about 10 µg/mL **(34)**, and therefore majority of AZ was considered to be present as free fraction in FaSSIF$_{rat, upper}$ (the saturated AZ solubility was 14.9 µg/mL). In addition, AZ is more lipophilic compound than GF **(34)**. Hence, AZ permeation across the upper small intestinal membrane in FaSSIF$_{rat, upper}$ might be still strongly limited by the UWL, even though UWL resistance might be to some extent reduced by micelles.

The difference in duration of saturated solubility between GF and AZ was observed (Figs 2 and 3). This might be caused by the difference in their dose, physicochemical property of particle surface (interaction with GI membrane) and dissolution rate. According to the report from Masaoka et al., it took about 60 minutes for the administered drug to reach the maximum concentration in the lower intestine **(19)**. However,

in this study, the drugs concentration in the lower intestine reached the saturated solubility at very early time. This is because that GF and AZ are low soluble, and therefore even if only the small amount of administered drugs reach the lower intestinal segment at early time, it is enough amount for low soluble drugs to show the saturated solubility.

$Fa_{suspension, \ 3h, \ cor.}$ of GF was calculated from $Fa_{suspension, \ 3h}$ and $Fh_{suspension}/Fh_{solution}$ at infinite time was calculated by using equations (10)–(12). It is considered that the values of $Fh_{suspension}/Fh_{solution}$ are different at each time point after oral administration. However, $BA_{suspension}$ was 10.6% and $BA_{suspension, \ 3h}$ was 7.6% (data not shown), and therefore, the absorption was almost completed by 3 h. Hence, the value of $Fh_{suspension}/Fh_{solution}$ at infinite time was considered to be close to that at 3 h.

Although it is also important to estimate $Fg_{suspension}/Fg_{solution}$ for evaluation of true $Fa_{suspension, \ 3h}$ of GF, the calculation is too difficult under non-linear pharmacokinetics. However, $Fg_{suspension}/Fg_{solution}$ was considered to be almost 1, because the luminal GF concentration easily reached the saturated solubility with the increase in the dose due to the low solubility of GF. In our previous study, we reported that the C_{max} value of the luminal GF concentration time-profile after oral administration of 2 mg/240 g of GF suspension to rats, under which the GF pharmacokinetics showed linearity, was about 60 μg/mL in the upper small intestinal lumen **(26)**. This GF concentration was almost half the GF saturated solubility (about 100 μg/mL) in the upper luminal fluid **(26)**. Although the GF dose used in this study (25 mg) was about 12.5 times higher than that used in previous study, the luminal GF concentration was only 2 times higher. Therefore, the $Fa_{suspension, \ 3h, \ cor.}$ value (20.8%) was considered to approach the true $Fa_{suspension, \ 3h}$. In AZ, $Fg \times Fh$ of AZSO in the first pass effect ($BA_{solution}$ of AZ) was 90% (Table 3), meaning after 100 % AZ was oxidized into AZSO in intestinal mucosa and liver, only 10 % of the AZSO was metabolized into albendazole sulfone or other metabolites. Hence, if there was non-linearity in AZSO metabolism after oral administration of AZ suspension, the influence on the calculation of *in vivo* Fa of AZ is considered to be negligible.

Fasted state simulated rat colonic fluid ($FaSSCoF_{rat}$) was prepared based on total bile acid and phospholipid concentrations (9.2 ± 4.0 mM and 0 mM, respectively.) and the pH to predict GF

absorption at 3 h in colon by using the equation for MAD ($Xa_{colon, \ 3h} = P_{colon} \times Cs_{colon} \times S_{colon} \times t_{colon}$). The pH of colonic fluid (6.6) was determined by insertion of pH spear (Nikko Hansen & Co., Ltd., Osaka, Japan) into the partly cut colonic lumen. Cholic acid (4.5 mM) and deoxycholic acid (4.5 mM) were selected as components of bile acids in $FaSSCoF_{rat}$, because these components account for about 20–35% of the bile acids in the region **(35)**, although taurocholic acid is the dominant component of bile acid in the rat small intestine **(36)**. The colonic permeability of GF dissolved in $FaSSCoF_{rat}$ (P_{colon}) and saturated GF solubility in $FaSSCoF_{rat}$ (Cs_{colon}) were 3.6 ± 0.51 cm/sec and 26.7 ± 0.58 μg/mL, respectively. GF concentration in colonic fluid at 6 h (17.1 ± 10.4 μg/mL, Fig 2) was similar to the saturated GF solubility in $FaSSCoF_{rat}$. Colonic lengths (15.4 ± 1.0 cm) were directly measured using a ruler, and the surface area in colon was calculated by using a radius of 0.25 cm (Cs_{colon}, 24.1 cm^2) **(20)**. In colon, the GF started to appear 1.5 h after oral administration (Fig. 2). Therefore, the duration time of the GF saturated solubility in colon was regarded as 1.5–3.0 h for estimation of the fraction GF absorbed from the colon over 3 h ($Fa_{colon, \ 3h} = Xa_{colon, \ 3h}/Dose$). As a result, the $Fa_{colon, \ 3h}$ was only 0.5%, in spite of that it is assumed GF concentration sustained the saturated solubility in the colon from 1.5h to 3 h, although GF did not actually reach saturated solubility in the colon until 3h. It may be due to much lower GF concentration in colon compared to those in upper and small intestine. AZ absorption in colon is also considered to be negligible until 3h. In the case of low soluble drugs, the contribution to colonic absorption might be low because of the low drug dissolution ability in colonic fluid.

The predicted Fa values at 3 h of GF and at 2.5h of AZ in the upper and lower small intestine were estimated as 5.2 and 12.4% in GF and 3.2 and 21.3% in AZ, respectively, using $FaSSIF_{rat, \ upper}$, and $FaSSIF_{rat, \ lower}$ (Table 4). This result showed that GF and AZ were mostly absorbed in the lower small intestine until 3 h after oral administration. When only $FaSSIF_{rat, \ upper}$ was employed for the prediction, the lower small intestine Fa value at 3 h was about half in GF (6.3%) and one third in AZ (7.9%) of the value estimated using $FaSSIF_{rat, \ lower}$. This was caused by the higher GF solubility in $FaSSIF_{rat, \ lower}$ than in $FaSSIF_{rat, \ upper}$ as well as longer residence time of drugs in lower small intestine. In consideration of these factors, evaluation of

solubility and permeability in lower intestinal region is much more important for prediction of oral absorption especially in low soluble compounds.

CONCLUSIONS

Simulated rat GI fluids reflecting the upper and lower small intestinal fluids were prepared to evaluate the regional differences in membrane permeability and solubility of GF and AZ, a model drug with poor water solubility. In addition, simulated colonic fluid was also developed to evaluate GF absorption in colon. Oral GF and AZ absorption in rats was predicted using these parameters and compared to actual absorption *in vivo*. Large regional differences were identified in oral absorption of model drugs, and the Fa values were predicted accurately. These results clearly indicated that use of fluids simulating not only the upper intestinal segment fluid, but also the lower segment fluid, was very important for reliable simulation of oral absorption of drugs with low aqueous solubility, because higher drug solubility and longer drug residence time in lower small intestine in addition to influence of regional difference in micelles concentration on drug intestinal permeability. This approach to Fa prediction using a range of simulated GI fluids will be very useful for oral drug development.

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