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Species Differences in the Dissolution and Absorption of Griseofulvin and Albendazole, Biopharmaceutics Classification System Class II Drugs, in the Gastrointestinal Tract

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Summary: It is well known that large differences exist in the bioavailability of orally administered drugs between species. Dissolution is the first step in the oral absorption of solid drugs. In this study, we measured the in vivo luminal concentrations of griseofulvin (GF) and albendazole (AZ), Biopharmaceutics Classification System (BCS) class II drugs, and the GF fraction absorbed (Fa) in rats. Then, we compared the GF Fa in rat with that in other species reported previously to evaluate differences in drug dissolution and oral absorption. The Fa of GF has been reported to decrease from 80% to 40% with an increase in the oral dose in dogs and humans, because the rate-limiting step for absorption shifts from dissolution to solubility. However, such non-linearity was not observed in rats that were administered doses in the same ranges as those in humans, and the Fa values in rats were higher than those in dogs or humans. The in vivo luminal concentration of GF after oral administration in rats was much higher than the saturated solubility of GF in fasted-state simulated dog (FaSSIF dog) or human intestinal fluid (FaSSIF human). Furthermore, oral administration of AZ showed similar tendencies of interspecies differences in dissolution and oral absorption.

Keywords: bile acids; dissolution; low soluble drug; oral absorption; regional difference; species difference

Introduction

Oral administration is the most convenient and common route for drug therapy because of good patient compliance. Currently, more than 60% of the marketed drugs are available as oral products such as tablets and capsules. Therefore, understanding the pharmacokinetics of the drug after oral administration is essential for drug development. At present, animals such as mice, rats, dogs, and monkeys are used to evaluate the pharmacokinetics of new drug candidates in preclinical studies, and the data from these studies are used to judge the safety and efficacy of the drug in humans before clinical studies. However, it is well known that large differences exist in the bioavailability (BA) of orally administered drugs between species.1) Species differences exist throughout the process of oral absorption of drugs from the intestinal lumen to the systemic circulation, including dissolution of the drug in the gastrointestinal (GI) tract, its permeation across the GI membrane, and metabolism in the small intestine and liver. Although species differences in drug permeation and metabolism have been widely investigated,2-5) little information is available about species differences in drug dissolution in the GI tract and the influence of these differences on drug absorption.

Bile acid and phospholipid concentrations in the GI tract are important factors that influence the solubility, dissolution rate, and oral absorption of drugs because of micellization.6-8) Under fasting conditions, the total bile acid concentrations in the upper jejunum of dogs (2.41–9.39 mM) are higher than those in humans (1.52–2.9 mM),9-11) while phospholipid concentrations are less than 0.2 mM in humans and less than 0.5 mM in dogs. However, 1 outlier was observed in each species (0.3 mM in humans and 8.12 mM in dogs) in this particular study.11) We have previously reported that total bile acid and phospholipid concentrations in the upper intestinal fluid were much higher in rats (bile acid concentration, about 51 mM and phospholipid concentration, 3.7 mM) than in either dogs or humans,12) and that the saturated solubility of griseofulvin (GF) in the upper jejunal fluid of rats was also much higher than that in fasted-state simulated intestinal fluid of dogs (FaSSIF dog) or fasted state-simulated human intestinal fluid (FaSSIF human). These data suggest that drug dissolution behavior in the GI tract is probably different between the species.
Many of the newly synthesized drug candidates are highly lipophilic. The rate-limiting step for intestinal absorption of highly lipophilic drugs is their dissolution in the intestinal fluid. Therefore, the oral absorption of compounds can be strongly influenced by their dissolution behavior in the GI tract, and it is extremely important to identify species differences in drug dissolution in the GI tract and the influence of these differences on drug absorption.

In this study, we compared the fraction absorbed (Fa) and area under the curve (AUC) after oral administration of GF and albendazole (AZ), which are Biopharmaceutics Classification System (BCS) class II drugs (i.e., drugs with low solubility and high permeability), in rats with those in other species reported in literature.

System (BCS) class II drugs (i.e., drugs with low solubility and high permeability), in rats with those in other species reported previously to identify species differences in oral absorption. In addition, we measured the luminal concentrations of GF and AZ in rats and used the GF and AZ solubility data in FaSSIFhuman and FaSSIFdog to elucidate species differences in drug dissolution.

Materials and Methods

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

In vivo oral absorption study in rats: GF and AZ powders were suspended in 0.5% methylcellulose solution at 1 or 2 mg/mL for GF and at 1.2 or 2.4 mg/mL for AZ. GF solution was prepared by dissolving GF powder in 40% polyethylene glycol 400 (PEG 400) and 10% dimethyl sulfoxide (DMSO) solution at a concentration of 0.45 mg/mL. To prepare the AZ solution, AZ powder was dissolved in 30% PEG 400 and 45% DMSO solution at 0.3 mg/mL. Then, the drugs were administered to male Wistar rats (Japan SLC, Hamamatsu, Japan) at 1 mL/240 g body weight by oral gavage (GF dose: 4.17, 8.33, or 1.875 mg/kg and AZ dose: 5, 10, or 1.25 mg/kg). Male rats weighing 230–250 g were randomly assigned to each experimental group, and all rats were fasted overnight before drug administration and remained in the fasting condition during the experiments. Blood samples were obtained from the femoral artery periodically via a cannula. Plasma obtained by centrifugation was deproteinized by acetonitrile precipitation. After centrifugation, the supernatant was evaporated and then resuspended in the high-performance liquid chromatography (HPLC) mobile phase for GF and 20% DMSO for AZ and AZSO, respectively. Then, the drug concentration of each sample was analyzed using HPLC.

Measurement of luminal concentrations of GF and AZ: After oral administration of GF and AZ suspensions at the same doses as those used in the oral absorption study, the rats were killed at each designated time point. Then, the abdomen was immediately opened to sample luminal fluid from the stomach and the upper (from a site 5–30 cm distal to the stomach) and lower jejunum (from a site 10–30 cm proximal to the cecum) by using a micropipette. The samples were instantly filtered through 0.65-µm hydrophilic polyvinylidene fluoride (PVDF) centrifugal filter units (Ultrafree®; Millipore Corporation, Billerica, MA). The amount of each filtrate was weighed and calculated by assuming the relative density of the samples to be 1. The filtrates were diluted with water and 25% DMSO for GF and AZ, respectively. Adsorption of AZ to the microtube surface because of high hydrophobicity can be avoided by adding DMSO to the solution.

The GF and AZ concentrations in each sample were quantified using HPLC. All animal studies were performed in accordance with the Guidelines for the Care and Use of Laboratory Animals of the Committee for Animal Experiments of Hiroshima International University.

Solubility measurement of AZ in Japanese Pharmacopoeia (JP)-1 solution and FaSSIFhuman: The saturated solubility of AZ was determined after 24 h of equilibration in JP-1 solution (pH 1.2) and FaSSIFhuman in an incubator at 37°C. Excess AZ was suspended in JP-1 solution and FaSSIFhuman. The suspensions were vortexed, and aqueous samples were filtered through 0.65-µm PVDF centrifugal filter units for JP-1 solution and 0.45-µm cellulose membranes for FaSSIFhuman after 24 h. In the case of FaSSIFhuman samples, the inside of each syringe was thoroughly washed with each sample, and the first 1 mL was discarded to avoid loss of AZ from the sample because of adsorption. The amount of each filtrate was weighed and calculated by assuming the relative density of the filtrate to be 1. The samples were then diluted with 20% DMSO solution.

Calculation of Fa(suspension): The Fa(suspension) of GF and AZ was calculated by comparing AUC0–∞suspension with AUC0–∞solution after oral administration of GF and AZ as either a suspension or a solution.

Rel. BA(suspension/solution) = \frac{\text{AUC}_{0-\infty,\text{suspension}} \times D_{\text{solution}}}{\text{AUC}_{0-\infty,\text{solution}} \times D_{\text{suspension}}} \times 100

The Fa(suspension) of GF and AZ was regarded as 1 because the drugs were administered as a solution and could be completely absorbed from the GI tract because of their high permeability. We assumed linear kinetics of drug metabolism in the GI mucosa (Fg) and the liver (Fl) after both administrations. Therefore, the relative BA of suspension to solution (Rel. BA(suspension/solution)) gives the Fa(suspension).

HPLC analysis: The concentration of GF in samples obtained from the in vivo oral administration and in vitro luminal concentration study, and AZ samples obtained from the in vitro solubility and in vivo luminal concentration study were determined using an HPLC system, which consisted of an HPLC pump (LC-20AD; Shimadzu Corporation, Kyoto, Japan) and a UV detector (SPD-20A; Shimadzu Corporation). An analytical column (YMC-Pack Pro C18; 150 × 6.0 mm I.D.; YMC Co., Ltd., Kyoto, Japan) was used at 40°C. 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, Santa Clara, CA) at 40°C. 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

Plasma GF and AZSO concentrations: The plasma concentration-time profiles after oral administration of GF and AZ to rats as either a suspension or solution are shown in Figure 1. After intestinal absorption, AZ is rapidly oxidized into its pharmacologically active metabolite, AZSO, in the mucosal cells and the liver. Thus, the AZSO concentration is used for the evaluation of
oral AZ absorption. The maximum plasma concentration (\(C_{\text{max}}\)) values after oral administration of 4.17 and 8.33 mg/kg of GF suspension were about 0.21 \pm 0.10 and 0.36 \pm 0.19 \mu g/mL, respectively. The \(C_{\text{max}}\) of AZ increased from 0.93 \pm 0.04 to 1.54 \pm 0.68 \mu g/mL with an increase in the dose from 5 to 10 mg/kg. The time to \(C_{\text{max}}\) (\(T_{\text{max}}\)) values after oral administration of GF (1 h) and AZSO (2 h) solutions were less than those after administration of the suspension (GF, 2 h and AZSO, 3 h).

Comparison of the Fa and AUC values between species:
The Fa and AUC\(_{0\rightarrow\infty}\) values after oral administration of GF and AZ in a solid dosage form to rats, dogs, and humans are summarized in Tables 1 and 2. The data for dogs and humans were obtained from previous studies.19,20,23 Although the dosage form in which GF was administered to humans is not clearly stated in the previous studies, GF and AZ were administered as a powder in capsules to dogs and humans, respectively. The human dose (mg/kg) was calculated by assuming the weight of humans to be 60 kg.

The AUC\(_{0\rightarrow\infty}\) values after oral administration of 4.17 or 8.33 mg/kg of GF suspension and 1.875 mg/kg GF solution were 0.79 \pm 0.23, 1.51 \pm 0.20, and 0.39 \pm 0.10 \mu g/mL, respectively. The Fa values calculated from the AUC\(_{0\rightarrow\infty}\) values were 91 \pm 27% at a dose of 4.17 mg/kg and 88 \pm 12% at a dose of 8.33 mg/kg. The Fa values at low doses of GF in humans (80%) and dogs (85%) decreased to 40% and 47%, respectively, after administration of a high dose of GF. However, the Fa values were nearly identical for both low and high doses of GF in rats.

The AUC\(_{0\rightarrow\infty}\) values of AZSO after oral administration of 5 and 10 mg/kg of AZ as a suspension and 1.25 mg/kg as a solution were 5.17 \pm 0.86, 9.87 \pm 5.63, and 3.72 \pm 0.62 \mu g/mL, respectively. Although the AUC\(_{0\rightarrow\infty}\) value at the high dose was about 2 times higher than that at the low dose in rats, the AUC\(_{0\rightarrow\infty}\) value was unchanged upon increase in the dose from 5 to 10 mg/kg in humans. The Fa values of GF and AZ in rats were higher than the corresponding values in dogs and humans.

Luminal GF and AZ concentrations: The luminal GF and AZ concentrations in rats were measured, and these concentrations were compared with the saturated solubility of GF and AZ in FaSSIFhuman and FaSSIFdog (Figs. 2 and 3). In our previous study, we have reported the saturated solubility of GF in FaSSIFhuman and FaSSIFdog as 13.1 \pm 0.28 and 16.0 \pm 0.86 \mu g/mL, respectively.12 The saturated solubility of AZ in FaSSIFhuman was 0.68 \pm 0.07 \mu g/mL.

The concentration of GF in the stomach at 0.5 h after oral administration of 4.17 and 8.33 mg/kg of GF as a suspension was about 13 \mu g/mL, and the concentration decreased gradually with time. The GF concentrations in the upper and lower jejunum at the 8.33 mg/kg dose were higher at each time point than the corresponding concentrations at the 4.17 mg/kg dose. The average concentrations of AZ at each time point in the stomach were 63.2 \mu g/mL at the 5 mg/kg dose and 99.2 \mu g/mL at the 10 mg/kg dose. These concentrations were much higher than those in the upper or lower jejunum. AZ has nitrogen with a pKa of 2.8, which shows basic properties at the third position of the imidazole ring.24 The pH values of the gastric fluid and the upper and lower jejunum of the rats were 1.4–1.9 and 6.7–7.4, respectively.12 AZ was positively ionized in the stomach; therefore, its concentration increased drastically. The saturated solubility of AZ in JP-1 solution (pH 1.2) was about 510 \mu g/mL. This finding further supports the increased solubility of AZ in the stomach. The luminal concentrations after oral administration of 10 mg/kg of AZ were higher than the concentrations in the upper and lower jejunum after administration of 5 mg/kg of AZ.

Discussion

In this study, we measured the luminal concentrations of GF and AZ, and compared the Fa values of the 2 drugs in rats with those reported for other species to evaluate differences in dissolution and oral absorption. In addition, we discussed the differences in drug dissolution according to the anatomical region.

Takano et al. established a method for estimating the oral absorption behavior of poorly water-soluble drugs by elucidating the rate-limiting steps of oral absorption.21,25,26 They suggested that if the luminal drug concentration is low (sink condition), drug absorption is limited by the dissolution rate, i.e., oral absorption is improved by a reduction in particle size. Conversely, if the luminal
drug concentration increases and approaches saturated solubility (non-sink condition), the absorption is limited by saturated solubility and not by dissolution rate, i.e., oral absorption does not improve with reduction in particle size, and the absorbed amount does not increase despite administration of a high dose.

The Fa and AUC\(_{0,\infty}\) values after oral administration of GF and AZ in rats, dogs, and humans are shown in Tables 1 and 2. The Fa values of GF have been reported to decrease from 80% to 40%, and the AUC\(_{0,\infty}\) values of AZSO are unchanged with an increase in the oral doses of GF and AZ in humans. The Fa values of GF in dogs follow a non-linear pattern even at a much lower dose range (0.2–2 mg/kg) than that reported in humans, which indicates that the rate-limiting step for drug absorption shifted from dissolution to solubility and therefore the dissolved and absorbed amounts did not increase despite administration of a high dose. However, such non-linearity was not observed in the Fa values of GF and AZ in rats, and these values were higher than those in dogs or humans. This is because the total bile acid and phospholipid concentrations in the GI tract of the rats were much higher than those in dogs or humans.\(^{12}\) Thus, these data indicate that the drugs with low solubility dissolve more poorly in the GI tract in humans than in rats; however, the dissolution in humans is better than that in dogs.

The AUC\(_{0,\infty}\) values in rats at 8.33 mg/kg of GF and 10 mg/kg of AZ were almost 2 times higher than those at 4.17 mg/kg of GF and 5 mg/kg of AZ. Therefore, we suspected that nonlinear metabolism did not occur in the GI mucosa and the liver, and thus the in vivo Fa values calculated on the basis of the AUC\(_{0,\infty}\) of both the suspension and solution were accurate. We measured the levels of intact AZ by using HPLC at the same time as AZSO quantification (data not shown). The HPLC conditions for AZ have been reported previously.\(^{27}\) The first-pass effect on AZ is almost 100%, which indicates that the parent compound is present at a very low concentration.\(^{28}\) In the present study, AZ was detected at 4–5 time points in the plasma samples, and the AUC\(_{0,\infty}\) values were calculated from the plasma AZ concentration-time profile. In addition, the AUC\(_{0,\infty}\) values of AZ at a dose of 10 mg/kg (0.156 ± 0.07 µg·h/mL) were 2 times higher than those at a dose of 5 mg/kg (0.074 ± 0.03 µg·h/mL). Thus, AZ showed linear metabolism within the tested dose range.

The in vivo luminal concentrations of GF and AZ in rats were measured and were compared to the saturated solubility of GF and AZ in FaSSIF\(_{\text{human}}\) and FaSSIF\(_{\text{dog}}\) (Figs. 2 and 3). The luminal concentrations of GF and AZ in rats were about 2 times higher with the high doses (8.33 and 10 mg/kg, respectively) than with the low doses (4.17 and 5 mg/kg, respectively). The absorption was limited by the dissolution rate and not by the saturated solubility (linearity in dissolution was sustained). The saturated solubility of GF in the upper and lower jejunal fluid of the rats was 99–146 and 153–260 µg/mL, respectively.\(^{12}\) The C\(_{\text{max}}\) values of the luminal GF concentration-time profile at a dose of 8.33 mg/kg were greater than the saturated solubility (53.3 ± 21.1 µg/mL in the upper jejunum and 84.2 ± 66.2 µg/mL in the lower jejunum). Therefore, increased amounts of GF can dissolve in the intestinal fluid with an increase...
in the GF dose. The saturated solubility of the 2 drugs in FaSSIFhuman and FaSSIFdog was much lower than their concentrations in the upper jejunal fluid of the rats. This is because of the differences in the total bile acid and phospholipid concentrations between FaSSIFhuman, FaSSIFdog, and rat upper jejunal fluid in vivo. FaSSIFhuman and FaSSIFdog consist of 3 and 5 mM sodium taurocholate and 0.75 and 1.25 mM egg lecithin, respectively, and the upper jejunal fluid of rats consist about 50 mM total bile acid and 3.7 mM phospholipids.\textsuperscript{12,17,18,29} Although the saturated solubility of GF in FaSSIFhuman was lower than that in FaSSIFdog, GF showed a nonlinear absorption pattern in dogs at a dose range lower than that in humans. 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 rate of permeation from the GI membrane, the absorption of the drug is limited by solubility.\textsuperscript{21,25,26} One of the reasons for this may be the lower volume of luminal fluid and/or permeability in dogs. Further studies are required to clarify this discrepancy.

In the case of BCS class II drugs, the Fa-time course is thought to determine the dissolution rate of drugs in the GI tract because the rate-limiting step for oral absorption is dissolution, because of the low solubility and high permeability of the drug. The BA time-course was calculated using a deconvolution method by using plasma concentration after oral and i.v. administration of GF, and the Fa-time course was obtained by dividing the BA\(\text{suspension,}\)\(t\) by the oral BA of the GF solution (BA\(\text{solution}\)) (Fig. 4).

\[
\text{BA}_{\text{solution}} = \frac{\text{AUC}_{0-\infty,\text{solution}}}{\text{AUC}_{0-\infty,\text{i.v.}}} \times \frac{\text{D}_{\text{i.v.}}}{\text{D}_{\text{solution}}} \times 100
\]

\[
\text{Fa}_{\text{suspension,}\text{t}} = \frac{\text{BA}_{\text{suspension,}\text{t}}}{\text{BA}_{\text{solution}}} \times 100
\]

Intravenous data (dose, 2 mg/kg) were used from a study by Fujikoa et al.\textsuperscript{30} The equation describing the plasma concentration was as follows: C\(p\) (\(\mu\)g/mL) = 0.56e\(^{-24.26t}\) + 0.76e\(^{-1.74t}\), and the AUC\(_{0-\infty}\) was 0.47 ± 0.08 \(\mu\)g·h/mL. The absorption rates were almost identical at both 4.17 and 8.33 mg/kg of GF administered as a suspension in rats. The linearity in dissolution was verified again by using the results of this experiment.

Information about in vivo luminal drug concentration after oral administration is very limited. Although in vivo luminal concentration-time profiles of fluorescein isothiocyanate (FITC)-dextran (FD-4) and BCS class I and III drugs after oral administration as a solution have been published,\textsuperscript{31} to our knowledge, this is the first study in which the in vivo concentrations of the dissolved drug in the luminal fluid have been reported after oral administration of BCS class II drugs in suspension form (Figs. 2 and 3). According to Masaoka et al.,\textsuperscript{31} FD-4 was almost completely emptied from the stomach within 1 h after ingestion, but the majority of GF and AZ remained after 1 h. The FD-4 concentration in the upper jejunum rapidly reached the \(C_{\text{max}}\) after 0.17 h. On the other hand, the GF and AZ concentrations in the upper jejunum reached the \(C_{\text{max}}\) at 0.5–1.5 h. This late transit rate with oral administration of GF and AZ suspensions compared to that with FD-4 solution may be because of adsorption of drug particles onto the GI mucosa.

In a previous study, we showed that saturated solubility of GF in the lower jejunum was higher than that in the upper jejunum.\textsuperscript{12} In addition, we observed a higher luminal concentration of GF in the lower jejunal fluid in vivo with both GF doses (Fig. 2). This is because the total bile acid concentration in rats is 2 times higher in the lower jejunum than in the upper jejunum.\textsuperscript{12} These data clearly indicate that the dissolution of drugs with low solubility in the GI tract differs largely according to the anatomical region. Therefore, considering the longer mean residence time of drugs in the lower region of the GI tract than in the upper region\textsuperscript{31,32} and the superior dissolution in the lower region, evaluation of drug dissolution in the lower region is more important to evaluate and/or predict the oral absorption of drugs with low solubility. We did not observe a regional difference in the luminal concentration of AZ between the upper and lower jejunum. AZ dissolved very efficiently in the gastric fluid because of its ionization; therefore, it is thought that entry of gastric fluid containing a very high concentration of AZ into the duodenal fluid increases the luminal concentration of AZ in the upper jejunum.

In conclusion, we identified species differences in both dissolution and oral absorption of BCS class II drugs. On the basis of our results, we conclude that drugs with low solubility dissolve more poorly in the GI tract in humans than in rats; however, the dissolution in humans is better than that in dogs. Oral absorption in humans may not be readily predictable using data obtained from rats or dogs because of the large species differences in drug dissolution in the GI tract. Considering that many drug candidates with low solubility have been synthesized, our findings may be very useful in the development of oral drug products.

References


