Forecasting the Oral Absorption Behavior of Poorly Soluble Weak Bases Using Solubility and Dissolution Studies in Biorelevant Media

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INTRODUCTION

The rate at which a drug goes into solution is an important determinant of drug absorption from the gastrointestinal tract. Factors that are important to the kinetics of drug dissolution, as identified by the Nernst-Brunner and Levich modifications of the Noyes-Whitney model (1–3), are the physicochemical properties of the compound itself such as pKa, solubility, crystalline energy and specific surface area, and certain aspects of the prevailing conditions in the gastrointestinal (GI) tract. The physiological parameters that can play an important role include pH, surface tension, solubilization, buffer capacity, and the volume of the lumenal contents. These parameters not only change following the ingestion of food, but also can vary widely with position in the GI tract.

Amphiphilic bile components including bile salts and lecithin, the concentration of which increase following a meal (4), have been shown to increase the *in vitro* dissolution rate for numerous poorly soluble compounds (5,6) either by an increase in solubility via micellar solubilization (at concentrations above the critical micelle concentration) and/or by the enhancement of the wettability of the compound.

Dipyridamole, BIMT 17 BS and BIBU 104 XX (Fig. 1), were chosen as structurally diverse examples of poorly soluble weak bases each displaying a dissolution-rate limited component to their absorption behavior, with intrinsic solubilities of 0.008, 0.0075, and 0.0028 mg/ml, respectively (Table I). The pKa values of the compounds, 6.4 (dipyridamole), 6.03 (BIMT 17 BS), and 5.8 (BIBU 104 XX) suggest that the dissolution characteristics could vary considerably during transit through the GI tract. Furthermore, the relatively high log D values for both dipyridamole (3.95 at pH 7.0) and BIMT 17 BS (3.3 at pH 7.4) indicate that the dissolution of these compounds may be influenced by the solubilizing effects of bile salts and lecithin. The lower log D value for BIBU 104 XX (2.3 at pH 7.4) suggests a lesser solubilization effect.

In the present study the aim was to assess the potential of *in vitro* solubility and dissolution studies using physiological relevant GI media to predict the *in vivo* absorption behavior of the aforementioned three poorly soluble weak bases.

MATERIALS AND METHODS

Materials

Dipyridamole powder (lot 170755), BIMT 17 BS powder (lot 9701-P), and BIBU 104 XX (lot 740097) powder were obtained from Boehringer Ingelheim Pharma KG, Biberach an der Riss, Germany. Table I lists the physicochemical properties of the three compounds.

Sodium taurocholate (NaTC) 98% pure (lot 15H5001) was purchased from Sigma-Aldrich Chemie GmbH (Deisenhofen, Germany). Egg-phosphatidylcholine (99.1% pure), lots 12091-1 and 105013-1, was generously donated by Lipoid GmbH (Ludwigshafen, Germany). Potassium dihydrogen phosphate, sodium dihydrogen phosphate, and potassium chloride, all analytical grade, were purchased from E. Merck (Darmstadt, Germany). All other chemicals were analytical grade or equivalent and purchased commercially.

Methods

Composition of the Various Media

The solubility of the drug powders was examined as a function of pH over the range typically encountered in the fasted stomach and in the proximal intestine in humans. McIlvaine buffer was prepared in the range pH 2.5 to 6.5 using citric acid monohydrate and disodium hydrogen phosphate. A hydrochloric acid buffer containing potassium chloride was used to study solubility at pH 2. The ionic strength of all media was held constant at 0.1 M by adjustment with sodium chloride. The final adjustment of pH was made using either 1M HCl or NaOH using a standardized pH meter (Orion, Boston, Massachusetts).

The solubility and dissolution characteristics were also examined in fasted state simulated intestinal fluid (FaSSIF) containing 3 mM NaTC and 0.75 mM lecithin in a pH 6.5 phosphate buffer and in fed state simulated intestinal fluid (FeSSIF) containing 15 mM NaTC and 3.75 mM lecithin in a pH 5.0 acetate buffer. The buffer systems and other components were designed to simulate average conditions in the proximal small intestine under both fasting and fed conditions (7). In addition, the dependency of solubility on the bile salt and lecithin concentration was examined using a pH 6.5 phosphate buffer containing NaTC in the concentration range 0–10 mM and in pH 5.0 acetate buffer containing NaTC in the 0–20 mM range (lecithin was included in a NaTC to lecithin ratio of 4 to 1). This was to simulate the range of bile salt and lecithin concentrations that can be anticipated during the pre- and postprandial states in the proximal small intestine (4).

Solubility Analysis

For the determination of the saturation solubility, excess amounts of drug were added to 20 ml of solvent in scintilla-

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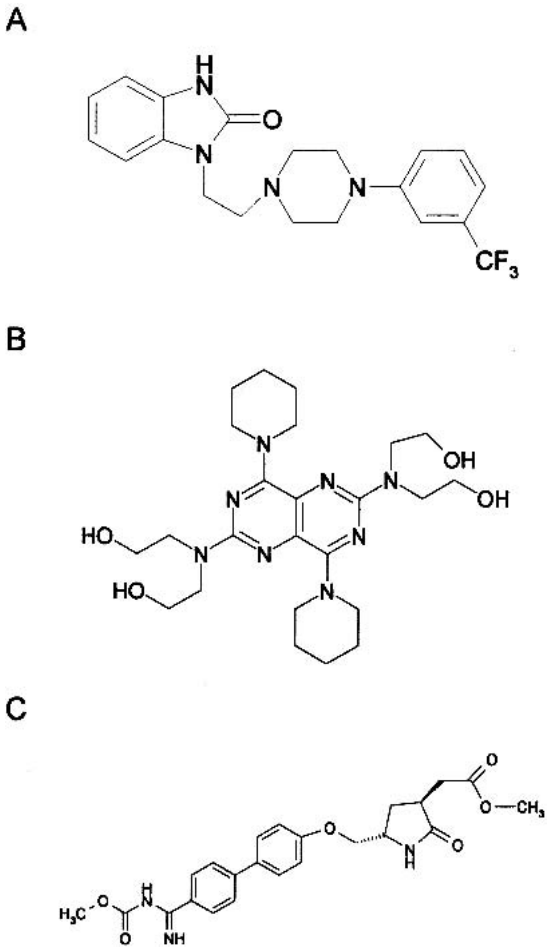

Fig. 1. Structures of the compounds studied: (A) BIMT 17 BS, (B) dipyridamole, and (C) BIBU 104 XX.

tion vials and placed in an Heraeus incubator shaker (Heraeus Instruments, Hanau, Germany) and maintained at 37°C until equilibrium was reached (24–48 h). All solubilities were determined in triplicate.

Dissolution

For all dissolution tests, the USP Apparatus 2 (paddle method) was used, employing a volume of 500 ml dissolution media at a temperature of 37°C ± 0.5°C. The dissolution characteristics of the compounds were examined under sink conditions by adding directly into the dissolution vessel an amount of pure drug powder equivalent to 20% of the solubility value in the respective media. For BIBU 104 XX, a paddle speed of 75 rpm was used, for dipyridamole and BIMT 17 BS the paddle speed was 100 rpm. An Erweka model DT80 (Heusenstamm, Germany) was used for all of the dissolution studies.

Table I. Physicochemical Properties of the Compounds Studied

Compound	Aqueous solubility (mg/ml)[a]	Log D[b]	Melting point	pKa	Mol. weight g · mol^{-1}
Dipyridamole	0.008	3.95 (pH 7.0)[c]	163°C	6.4	504.6
BIMT 17 BS	0.0075	3.3 (pH 7.4)	161°C[d]	6.03	390.41
BIBU104 XX	0.0028	2.3 (pH 7.4)	197°C[d]	5.8	439.5

[a] Determined experimentally at 37°C.
[b] Octanol/water distribution coefficient.
[c] (15).
[d] 10°C · min^{-1} heating rate.

Sampling Procedure

For both the solubility and dissolution studies, 5 ml samples were periodically withdrawn and filtered, and the same volume of blank, prewarmed medium was replaced into the vessel, as described previously (7). The samples were appropriately diluted prior to analysis of drug concentration by high-performance liquid chromatography (HPLC). No significant loss of drug due to adsorption to either the sampling device or filter was detected for any of the three compounds. For the dissolution studies, concentrations were corrected for the sample amount of drug removed at the corresponding time.

HPLC Analysis

Drug concentrations were determined using a HPLC system consisting of an autosampler (model ISS 101, Perkin Elmer, Norwalk, Connecticut), a Shimadzu C-R5A integrator (Shimadzu, Kyoto, Japan), and a Merck Hitachi pump (model L-7110) and Spectroflow 757 absorbance detector (Merck Hitachi, Darmstadt, Germany). An injection volume of 100 μl was used for each analysis.

For both dipyridamole and BIMT 17 BS the analyses were performed on a LiChrospher® 100 RP-8, 5 μm column (Merck, Darmstadt, Germany) using a mixture of 64.9% methanol, 34.9% 0.01N HCl, and 0.2% DEA as mobile phase with a flow rate of 1.0 ml/min. For dipyridamole, the detection wavelength was set at 284.5 nm, and the compound eluted at approximately 4.5 m. BIMT 17 BS typically eluted at 4 min at a detection wavelength of 280 nm.

The mobile phase used for BIBU 104 XX consisted of a mixture of 55% acetonitrile and 45% 0.05M ammonium formate and was pumped at a flow rate of 1.0 ml/min. BIBU 104 XX had an approximate retention time of 3.6 min using a Prodigy ODS (3), 250 × 4.6 mm (interior diameter) 5 μm column (Phenomenex, Aschaffenburg, Germany) and detection wavelength set at 300 nm.

A seven-point calibration curve was prepared for each drug and linearity (r^2 not less than 0.999) was observed in the 0.1–50 mg/L concentration range. The data obtained following the HPLC analysis were transferred to Excel™ (Microsoft, Redmond, Washington) for subsequent data analysis.

RESULTS

Solubility Studies

In the range pH 2 to 6.5, all three compounds showed an approximately linear relationship between the logarithm of the solubility and pH (Fig. 2). Over this range, the solubility is dramatically increased by a factor of 820 for BIBU 104 XX, 3300 for dipyridamole, and by almost 9200 for BIMT 17 BS.

An increase in bile component concentration at a given pH resulted in substantial increases in solubility for both dipyridamole and BIMT 17 BS, with the enhancement being more pronounced for the latter. Increasing bile components

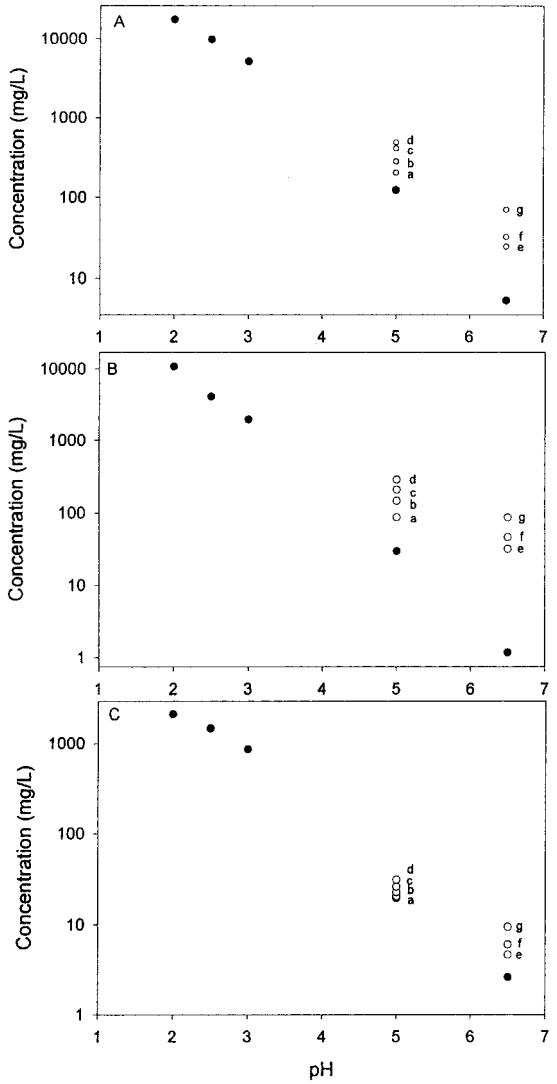

Fig. 2. Mean solubility as a function of pH and bile salts/lecithin concentration for (A) dipyridamole, (B) BIMT 17 BS, and (C) BIBU 104 XX. NaTC/lecithin concentrations: a. 5 mM/1.25 mM; b. 10 mM/2.5 mM; c. 15 mM/3.75 mM; d. 20 mM/5 mM; e. 3 mM/0.75 mM; f. 5 mM/1.25 mM; g. 10 mM/2.5 mM.

from none to 10 mM NaTC/2.5 mM lecithin at pH 6.5 resulted in a 75-fold increase in BIMT 17 BS solubility and a 13-fold increase in dipyridamole solubility. Corresponding values at pH 5.0, 20 mM NaTC/5 mM lecithin were tenfold for BIMT 17 BS and fourfold for dipyridamole. For BIBU 104 XX the enhancement in solubility with an increase in bile salt and lecithin concentration was not as pronounced at either pH 5.0 (1.6-fold) or pH 6.5 (3.6-fold) compared with dipyridamole and BIMT 17 BS.

Table 2 shows the dose to solubility (D/S) ratio for the three compounds determined in pH 2 and pH 5 buffer simulating fasted and fed state conditions in the stomach and also in FaSSIF and FeSSIF. In pH 2 buffer the D/S ratio for dipyridamole (11 ml), BIMT 17 BS (5 ml), and BIBU 104 XX (14 ml) is significantly lower than in pH 5 buffer (approximately 1.6 l) for all three compounds. To compare with the volumes observed in the fasted stomach, one can use a basal volume of between 30 and 50 ml. However, because 200–250 ml of water is normally given with the dose in a pharmacokinetic study, one can anticipate that the total initial volume will be approximately 250 to 300 ml. After ingestion of a meal the volume in the stomach can be expected to be approximately 500 ml, which will remain relatively constant over a period of at least 2 hours after the meal (4).

Table II. Calculated Dose to Solubility Ratios for Dipyridamole, BIMT 17 BS and BIBU 104 XX under Various Conditions in the Upper GI Tract

		Stomach		Intestine	
	Dose	Fasted[a]	Fed[b]	Fasted[c]	Fed[d]
Dipyridamole	200 mg	11 ml	1.6 L	8 L	0.5 L
BIMT 17 BS	50 mg	5 ml	1.7 L	1.5 L	0.2 L
BIBU 104 XX	30 mg	14 ml	1.5 L	6.5 L	1.2 L

[a] pH 2 buffer.
[b] pH 5 buffer.
[c] FaSSIF.
[d] FeSSIF.

In FaSSIF the D/S values for dipyridamole and BIMT 17 BS are 8 and 1.5 l, respectively, and in FeSSIF they decrease to 0.5 and 0.2 l. For BIBU 104 XX the volumes decrease from 6.5 l in FaSSIF to 1.2 l in FeSSIF. Typical volumes in the small intestine after meals range between 1 and 1.5 l, but in the fasted state the volume is significantly smaller and is probably closer to the volume of fluids coadministered with the dose.

Dissolution Studies

Figure 3A shows the mean dissolution profiles for dipyridamole powder examined in FaSSIF and FeSSIF at 100 rpm under sink conditions. In FeSSIF, the medium simulating fed conditions in the duodenum, dissolution was quite rapid with almost complete dissolution occurring after 20 min. In the medium simulating fasting conditions in the duodenum (FaSSIF), the rate of dissolution was significantly lower, such that even after 2 h only 80% dissolution had occurred. In Fig. 3B the mean dissolution profiles for BIMT 17 BS at 100 rpm in FaSSIF and FeSSIF media are shown. The rate of dissolution in FeSSIF is relatively quick, achieving complete dissolution within 25 min. In contrast, the dissolution in FaSSIF is only complete after 120 min. As shown in Fig. 3C, the dissolution of BIBU 104 XX is also greater in FeSSIF than in FaSSIF. The enhancement in dissolution for all three compounds in FeSSIF indicates that the elevated bile salt and lecithin concentration combined with the lower pH value have an important influence on the dissolution rate.

DISCUSSION

To forecast the *in vivo* performance of a drug, it is important that the *in vitro* test should mimic the conditions *in vivo* as closely as possible. To achieve this, biorelevant GI media were used examine the solubility and dissolution characteristics of three poorly soluble weak bases to assist in making *in vivo* predictions. Other factors that may be important to the absorption process (e.g., intestinal permeability and GI metabolism) were not taken into consideration in the present study. Therefore, the current predictive approach is better

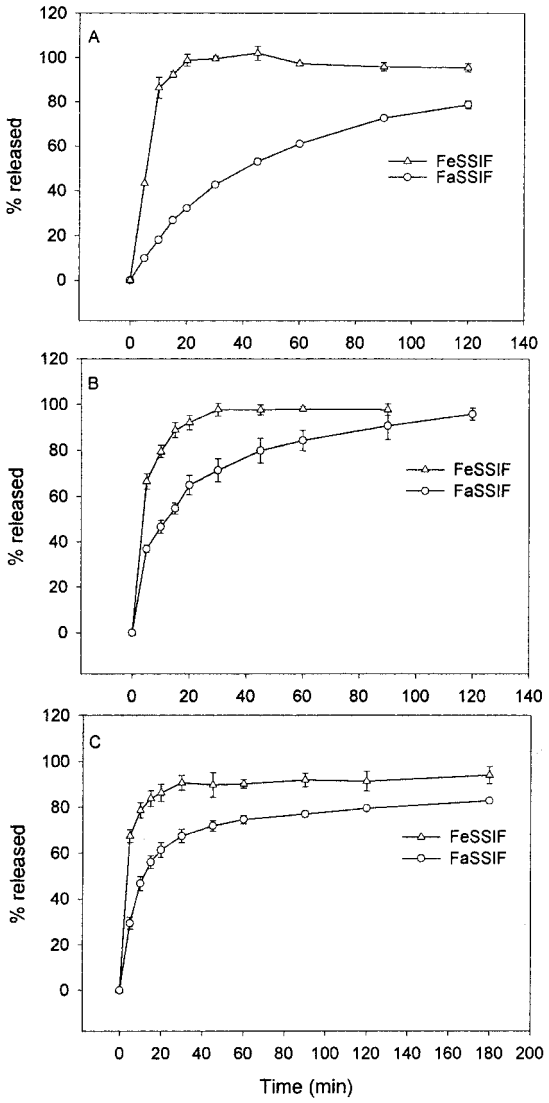

Fig. 3. Mean dissolution profiles in FaSSIF and FeSSIF under sink conditions for (A) dipyridamole, (B) BIMT 17 BS, and (C) BIBU 104 XX.

suited to making predictions for individual compounds than comparing behavior among multiple compounds.

As expected for weak bases in general, the solubility of dipyridamole was significantly enhanced by a reduction in pH. Significant differences therefore can be anticipated between the relatively acidic conditions in the fasted stomach, with pH values ranging between 1.2 and 2.5, compared with the less acidic conditions in the stomach after food or administration of gastric acid secretion blockers (pH 3–7), and in the fasting duodenum (pH 4.9–6.5) (8–10).

The effect of the GI environment on solubility and its potential effects on absorption can also be evaluated by assessing the dose to solubility (D:S) ratio. The solubility studies clearly illustrate that the acidic environment of the fasting stomach is an important site for the dissolution of the dipyridamole dose. In contrast, elevating the pH to 5, which may occur in the stomach following meal intake or administration of gastric acid secretion blockers, complete dissolution of the dose is not expected in the stomach due to the much higher volume of gastric fluids required. Based on the limited solubility in the FaSSIF medium, if the entire dipyridamole dose is not dissolved in the stomach there is unlikely to be enough capacity in the upper small intestine to ensure completion of the dissolution process. The direct influence of gastric pH on dipyridamole absorption has been clearly illustrated by Russell *et al.* (11), who showed that in subjects with an elevated gastric pH (normal subjects after famotidine pretreatment or achlorhydric individuals) the extent of absorption was significantly reduced compared to in subjects with low gastric pH (normal subjects without pretreatment or in achlorhydrics with coadministration of glutamic acid).

In the fasted state, gastric emptying is highly dependent on the motility phase at the time of drug administration. Because the stomach is the only site at which conditions are favorable for dipyridamole dissolution in the fasted state, variability in gastric emptying is likely to result in variability in dipyridamole bioavailability. In the fed state the importance of the stomach as the major site for dissolution will be compromised due to the elevated gastric pH. The higher bile salt and lecithin concentrations in the upper intestine, combined with a more favorable pH than in the fasted state, establishes this region of the GI tract as a potentially important site for dissolution that can at least partly compensate for the incomplete dissolution in the stomach. In light of the longer upper gastrointestinal transit in the fed state (12), the solubility and dissolution results indicate that absorption should be greater when dipyridamole is administered with food.

The *in vitro* predictions for the fed vs. fasted bioavailability of dipyridamole concurred with the results of a further *in vivo* study performed in healthy subjects (data on file, Boehringer Ingelheim Pharma KG). The influence of giving a meal 30 min prior to an oral 100 mg dose of dipyridamole as a suspension in nine healthy volunteers resulted in an approximately 12% increase in the area under the curve (AUC) (0–24 h) values compared with the corresponding value following administration in the fasted state (4.6 ± 2.2 fasted vs. 5.2 ± 1.6 μg.h/ml fed). Food delayed the rate of absorption, with an approximate 29% reduction in peak plasma concentrations from 1.6 ± 0.6 μg/ml to 1.2 ± 0.4 μg/ml and an increase in the Tmax values from 0.64 to 0.97 h, both of which can be explained in terms of the elevated residence time combined with the reduced solubility in the stomach. In an earlier study, Mellinger and Bohorfoush (13) also demonstrated an increase in dipyridamole absorption following food. In that study, a meal 2 h following dosing of a 25 mg tablet in four subjects resulted in an approximate 60% increase in Cmax levels compared with the levels observed in the control group. Interestingly, Mithani (14) has demonstrated the potential for dipyridamole to precipitate in the duodenum at pH and bile salt concentrations representative of the fasted state but not the fed state, indicating that precipitation in the small intestine may be a further explanation for the lower bioavailability observed under fasting conditions.

The solubility studies clearly illustrate the importance of pH on BIMT 17 BS dissolution. In the relatively acidic environment of the fasted stomach, it is predicted that dissolution of the dose will be complete. Not surprisingly, under situations of an elevated gastric pH, such as following meal intake, the solubility and dissolution will not be sufficient for complete dissolution of the dose to occur. Based on solubility studies in FaSSIF, it is anticipated that in the fasted state

conditions in the upper small intestine are not sufficient to achieve complete dissolution of the dose. Following conversion to the fed state however, the combination of a reduced pH and increased bile salt and lecithin concentrations in the intestine should lead to a 100% dissolution of the dose.

Taken together, the *in vitro* studies using the physiologically relevant media indicate that the oral bioavailability of BIMT 17 BS should be better if the drug is given in the fed state. These predictions concurred with results of an *in vivo* study in which a 50 mg dose of BIMT 17 BS (micronized) prepared as either a simple tablet or capsule formulation containing no surfactant excipients was administered to 22 subjects. The mean values of the area under the plasma concentration-time curve (from time zero to infinity) following a high fat breakfast increased by 36% for the capsule (1006 ± 603 vs. 1376 ± 645 ng.h/ml) and up to 40% for the tablet (1009 ± 475 vs. 1417 ± 757 ng.h/ml) (Boehringer Ingelheim Pharma KG, data on file). The Tmax value was delayed from approximately 0.5 h under fasting conditions to 4 h following administration with food, which may be explained by the slower gastric emptying rate.

The *in vitro* solubility studies indicated that for BIBU 104 XX the acidic environment of the fasted stomach is a prerequisite for the complete dissolution of the dose to occur. Incomplete dissolution will be expected in the fed state stomach because a much higher volume of gastric fluids is required. As expected from the pKa, the solubility in FaSSIF is also poor. Furthermore, the limited solubility in FeSSIF suggests that even with the higher bile salt and lecithin concentration, the capacity to dissolve the dose is insufficient to compensate for the incomplete dissolution in the fed stomach. The rather modest enhancement in solubility in the FeSSIF medium compared with FaSSIF may be related to the lower lipophilicity of this compound. In short, the stomach in the fasted state is the only site with favorable conditions for dissolution of BIBU 104 XX.

The prediction from the *in vitro* studies concurred with the results of an *in vivo* study performed in 12 healthy male subjects examining the influence of a meal following an oral 30 mg dose of BIBU 104 XX formulated as a simple tablet containing no surfactant excipients. Concurrent administration with a meal reduced AUC values by approximately 33% (1326 ± 357.2 vs. 883.3 ± 245.5 ng.h/ml) and caused a delay in the rate of absorption with an approximate 25% reduction in peak concentration (98.8 ± 39.8 vs. 72.1 ± 28.4 ng/ml) and a delay in Tmax from 1.75 to 2.5 h (data on file, Boehringer Ingelheim Pharma KG).

In conclusion, these three examples demonstrate that solubility and dissolution studies in biorelevant media are useful in predicting the *in vivo* performance of poorly soluble, weakly basic drugs.

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