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Estimating Human Drug Oral Absorption Kinetics from Caco-2 Permeability Using an Absorption-Disposition Model: Model Development and Evaluation and Derivation of Analytical Solutions for k_a and F_a

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ABSTRACT

Intestinal transcellular permeability (P_m), measured across cell lines such as Caco-2 cells in vitro, is often used for assessing oral drug absorption potential in humans. However, the quantitative link between in vitro permeability and apparent in vivo absorption kinetics, based on drug appearance in plasma, is poorly understood. In the current study, a novel absorption-disposition kinetic model that links traditional pharmacokinetic and mass transfer models was developed. Analytical solutions of k_a and F_a were deduced, and using Caco-2 permeability, F_a in humans was predicted for 51 structurally diverse compounds. Predicted F_a values were similar to and correlated highly with their corresponding experimental values with an average error of 1.88 ± 1.06% (−17 to 22%) and $r^2 = 0.934$. Simulated concentration profiles for 17 of 18 drugs corresponded to observed plasma concentration profiles in healthy volunteers. The equilibrium solution for k_a ($k_{a,eq}$) was found to be a key determinant of F_a, whereas under sink conditions, k_a is likely to be a determinant of plasma concentration kinetics. The current version of the model offers a quantitative approach for predicting human oral absorption kinetics from in vitro permeability. It also establishes, for the first time, a quantitative link between P_m and k_a and between $k_{a,eq}$ and F_a. This will facilitate better in vitro or in situ-in vivo correlations since it establishes a basis for incorporating permeability coefficients from the various experimental formats based on drug loss or appearance that are commonly used in the laboratory for permeability determination.

Oral administration is the most commonly used drug-dosing route. Therefore, the ability to predict the rate and extent of absorption of drug candidates after oral administration is crucial during the preclinical phase of development. Such knowledge complements high throughput drug screening and allows scientists to select the best drug candidates early in the drug development cycle. Drug absorption from the gastrointestinal (GI) tract is affected by many factors. Besides the physiological conditions of the GI tract (e.g., absorptive surface area, local pH, food effects, intestinal transit time, and passive intestinal permeability) and chemical properties of the drug (e.g., solubility, molecular size, and stability), intestinal transporters and enzymes are being increasingly implicated in controlling oral drug absorption (Martinez and Amidon, 2002). Because of this, the challenging task of quantitatively predicting oral drug absorption properties has attracted the attention of many scientists.

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So far, most predictive models have been developed based on intestinal transport mechanisms and physiological parameters or statistical/probabilistic analysis. Typically, the statistical models have been built using regression results from a training set (e.g., Zhao et al., 2001), and correlation results are then used to predict F_a for compounds outside of this training set. These models rely heavily on the selection of training set compounds and, consequently, could have limited predictive ability for compounds that are outside of the training set (i.e., out of the box). The transport/mechanistic models, such as the mass balance (Sinko et al., 1991, 1993), mixing tank (Dressman et al., 1984), and compartmental absorption and transit (CAT; Yu and Amidon, 1999) models, quantify F_a and k_d (the first-order disappearance rate constant in the intestine, also referred as intrinsic k_a) from in situ permeability measured in a single-pass intestinal perfusion system. Based on drug disappearance kinetics from the intestinal lumen, the analytical solutions of F_a and k_d were deduced from these models. Some of these models, such as the CAT model (Yu and Amidon, 1999), use k_d plus drug-

ABBREVIATIONS: GI, gastrointestinal; CAT, compartmental absorption and transit; PK, pharmacokinetic; ODE, ordinary differential equations; AUC, area under the curve; pred., predicted; exp., experimental.

specific disposition parameters to estimate drug absorption kinetics in plasma. F_a and k_a (the absorption rate constant in plasma) were determined from the disappearance kinetics of a drug in the intestinal lumen rather than from the appearance kinetics in plasma. These models assume that k_d is equal to k_a. Under this assumption, the prediction of k_a and F_a is accurate when k_a is similar to k_d or erroneous when k_a and k_d are significantly different. Increasingly, the physiological processes of intestinal cycling, enterohepatic cycling, and tissue accumulation of drugs are being implicated in absorption kinetics. This means that for many drugs k_a and k_d might not be equal. For instance, we have observed that the k_d and k_a of saquinavir were nearly 9-fold different in rat small intestine following a single-pass perfusion due to significant tissue retention (~5% of perfused drug amount found in tissue, whereas ~0.1% was recovered in the blood) (unpublished data). This has mechanistic implications since some experimental methods used to determine permeability are based on drug loss from the intestinal lumen (e.g., single-pass intestinal perfusion), whereas others are based on drug appearance (e.g., cultured cells in the Transwell format). Other experimental factors such as anesthesia may lead to a discrepancy between the measurement of permeability by loss or by appearance since blood flow and drug clearance is altered. For all of these reasons, there is a compelling need to update predictive oral absorption models so that the mechanistic link between permeability across the intestinal mucosa and in vivo absorption can be adequately reflected.

Pharmacokinetic (PK) models have been built based on drug disposition properties in the plasma compartment(s). Although extravascular PK models describe drug plasma concentration profiles well for orally administered drugs, these models are disconnected from drug kinetics at the absorption site. Even in the most sophisticated extravascular models (e.g., Wagner, 1993), drug kinetics in the intestinal lumen were treated as a black box. Because of this, k_a could only be determined from k_{el} (elimination rate constant) by using a deconvolution method (Wagner, 1975), and F_a could not be quantitatively determined.

Caco-2 cell permeability is often used as a screening tool for assessing drug oral absorption during the early stages of drug development. Despite some differences in transporter gene expression (Sun et al., 2002), Caco-2 cells possess many structural and functional similarities to normal human enterocytes, and Caco-2 permeability has generally correlated well with F_a for many drugs in humans (Yee, 1997). Hence, many scientists use Caco-2 permeability as a surrogate for human intestinal permeability. We have chosen this permeability determination format for the first application of this model since it is one of the most common formats used in laboratories today.

In this article, a novel absorption-disposition kinetic model that links the processes of gastric emptying, intestinal absorption, and plasma disposition was developed. Using this model, k_a, $k_{a,eq}$, and F_a were deduced from plasma kinetics. The absorption parameters in humans were predicted from Caco-2 permeability for 51 structurally diverse pharmaceutical compounds. From the predicted F_a and plasma concentrations in humans, the predictive ability of the model was assessed.

Materials and Methods

The Definition of Bioavailability and Its Related Terms. Oral bioavailability (F_{oral}) is defined as the extent of intact drug appearing in blood. F_{oral} is commonly reported in the literature by comparing the amount of drug in blood after oral administration with that after intravenous administration (i.e., absolute oral bioavailability). F_a is the fraction of drug taken up into the intestinal tissue by any mechanistic pathway (e.g., paracellular or transcellular). F_{gw} is the fraction of drug that survives intestinal metabolism and enters the portal vein. F_H is the fraction of drug that escapes hepatic metabolism and enters the hepatic vein intact. F_{FP} is the fraction of drug that escapes first-pass intestinal and hepatic metabolism ($F_{FP} = F_{gw} \times F_H$).

Model Development. Gastric emptying and intestinal transit can be approximately described by first-order kinetics under fasted conditions (Davenport, 1982). Drug absorption and elimination also generally follow first-order kinetics (Rowland and Tozer, 1995). Therefore, the pharmacokinetics of an orally dosed drug can be described using a three-compartmental kinetic model (Fig. 1) with one compartment each representing the stomach, intestine, and plasma. The M_s, M_i, and M_{pl} represent the amounts of drug in the stomach, intestine, and the amount absorbed as reflected in the plasma, respectively, and k_s, k_i, k_a, and k_{el} are first-order rate constants of gastric emptying, intestinal transit, absorption, and elimination from plasma, respectively. This model assumes that drug dissolution is not a rate-limiting step in drug absorption; drug absorption from the stomach is negligible and the body is a homogenous compartment. The corresponding ordinary differential equations (ODE) for this system are:

$$\frac{dM_s}{dt} = -k_s M_s \tag{1}$$

$$\frac{dM_i}{dt} = k_s M_s - (k_i + k_a) M_i \tag{2}$$

$$\frac{dM_{pl}}{dt} = k_a M_i F_{FP} - k_{el} M_{pl} \tag{3}$$

By solving the ODEs under the assumption that drug concentrations are below the solubility in the entire intestinal lumen and the initial conditions of $M_s = D_{oral}$, $M_i = 0$, and $M_{pl} = 0$, the drug concentration in the plasma compartment over time $(0, \infty)$ can be written as:

$$C_{pl} = \frac{AF_{FP}D_{oral}}{V_d}(Be^{-kst} - Ce^{-(ki+ka)t} - De^{-kelt}) \tag{4}$$

where V_d is the volume of distribution at steady-state, and

$$A = \frac{k_s}{k_i + k_a - k_s} \tag{5}$$

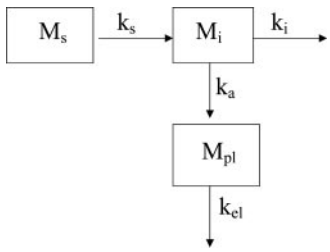

Fig. 1. Absorption-disposition kinetic model. M_s, M_i, and M_{pl} represent drug amount in the stomach, small intestine, and plasma, respectively. k_s, k_i, k_a, and k_{el} represent the rate constants of gastric emptying, intestinal transit, absorption, and elimination, respectively.

$$B = \frac{k_a}{k_{el} - k_s} \tag{6}$$

$$C = \frac{k_a}{k_{el} - k_i - k_a} \tag{7}$$

$$D = \frac{k_a(k_s - k_i - k_a)}{(k_{el} - k_s)(k_{el} - k_i - k_a)} \tag{8}$$

Analytical Solution of k_a. k_a is the first-order absorption rate constant based on appearance of drug in the systemic circulation. Drug absorption into the systemic circulation (i.e., peripherally measured in plasma) in the absence of elimination can be described as:

$$\frac{dM_{pl}}{dt} = k_a M_{pl} \tag{9}$$

First-pass metabolism is not assumed to be negligible in this case. Assuming that drug loss in the small intestinal lumen is due to absorption only, then drug disappearance from the intestine reflects the amount of drug absorbed and can be described by first-order kinetics or by Fick's Law under sink conditions:

$$\frac{dM_i}{dt} = -k_d M_i \tag{10}$$

and

$$-\frac{dM_i}{dt \times S} = P_m C_i = P_m \frac{M_i}{V_i} \tag{11}$$

where k_d is the disappearance rate constant, P_m is drug permeability across intestinal mucosa, and S is the absorptive surface area. By combining eqs. 10 and 11, k_d can be expressed as below (similar to the function derived by Ho and Higuchi, 1974):

$$k_d = \frac{P_m S}{V_i} \tag{12}$$

Because M_{pl} is the fraction of M_i due to the first-pass extraction ($F_{FP} = F_{gw} \times F_H$), the relationship between M_i and M_{pl} can be expressed as follows. Also, from this point on, M_{pl} and C_{pl} are referred to as the unchanged amount of drug and drug concentration in the plasma.

$$-\frac{F_{FP} \times dM_i}{dt} = \frac{dM_{pl}}{dt} \tag{13}$$

When combining eqs. 9, 11, and 13 and replacing M_{pl} with $C_{pl} \times V_c$, k_a is found to be:

$$k_a = \frac{P_m S}{V_c} \times \frac{F_{FP} C_i}{C_{pl}} \tag{14}$$

where V_c is the volume of distribution in well perfused organs. Using V_c instead of V_d is based upon the concept that initial drug distribution is limited by blood perfusion (Rowland and Tozer, 1995). Therefore, during the absorption phase, a drug is more likely distributed in well perfused organs, such as heart, kidney, lung, spleen, liver, and muscles rather than to peripheral tissues such as bone, fat tissue, and skin.

Equation 14 reveals that k_a has multiple analytical solutions, an equilibrium solution ($k_{a,eq}$) when $F_{FP}C_i/C_{pl} = 1$, and nonequilibrium solutions (k_a) when $F_{FP}C_i/C_{pl} \neq 1$. The equilibrium solution is independent of drug concentration changes in the intestine and plasma, whereas the nonequilibrium solutions are dependent upon the concentration ratio that varies over time. Since k_a is defined as a rate constant in this model, the equilibrium $k_{a,eq}$ was used in the following F_a derivation.

$$k_{a,eq} = \frac{P_m S}{V_c} \tag{15}$$

The Analytical Solution of F_a. Plasma AUC can be calculated using a property of Laplace transformation as follows:

$$AUC = \int_0^\infty C dt = \lim_{s \to 0} \bar{C} \tag{16}$$

Hence, from transformed ODEs of the absorption-disposition kinetic model,

$$AUC_{i.v.} = \frac{D_{i.v.}}{V_d k_{el}} \tag{17}$$

and

$$AUC_{oral} = \frac{k_{a,eq} F_{FP} D_{oral}}{V_d k_{el}(k_i + k_{a,eq})} \tag{18}$$

When eqs. 17 and 18 are combined, F_{oral} becomes:

$$F_{oral} = \frac{AUC_{oral} D_{i.v.}}{AUC_{i.v.} D_{oral}} = \frac{k_{a,eq} F_{FP}}{k_i + k_{a,eq}} \tag{19}$$

Since $F_{oral} = F_a \times F_{FP}$, F_a is:

$$F_a = \frac{k_{a,eq}}{k_i + k_{a,eq}} \tag{20}$$

Computational Methods. Fifty-one pharmaceutical compounds were selected as test compounds to evaluate the model based on the availability of their permeability data in Caco-2 cells and reported F_a in humans in the literature. F_a values are typically estimated from F_{oral} values. For example, Zhao and coworkers (Zhao et al., 2001) reported F_a values in humans depending on the degree of metabolism known to occur for any given drug. For drugs with minimal metabolism, F_{oral} was considered as F_a. However, for drugs with significant first-pass metabolism (i.e., when F_{oral} is less than F_a), they extrapolated F_a values from urinary and fecal excretion data. Specifically, F_a was estimated as percentage of dose excreted unchanged in the urine and as metabolites following i.v. and oral administration, percentage of metabolites in urine following oral and intravenous dosing, percentage of extraction of drug in bile after i.v. or oral dosing, percentage of accumulative excretion of drug in feces following i.v. or oral dosing, and total recovery of drug in urine and feces following oral and i.v. dosing. The drugs selected for this study cover a broad range of F_{oral} in humans. Among the selected drugs, 9 drugs have $F_{oral} < 20\%$, 10 drugs have F_{oral} ranging from 20 to 50%, 12 drugs have F_{oral} ranging from 50 to 80%, and 20 compounds have $F_{oral} > 80\%$ (Gilman et al., 1990). Since more than half of the drugs in this dataset are significantly metabolized on a first pass through the intestine and liver (Gilman et al., 1990), the drugs with F_a ranging from 20 to 50% is limited (i.e., only six drugs with $F_a < 20\%$ and eight drugs with $F_a = 20-80\%$).

For the $k_{a,eq}$ computation, the absorptive surface area of human intestine (S) was set to 200 m^2 (Snyder et al., 1975). Intestinal absorptive surface area is a dynamic parameter that depends on the method of measurement and the absorption properties of the drug. For example, a drug that is absorbed more slowly may have a higher net absorptive surface area since it will travel further down the villus compared with a drug that is absorbed more quickly. The effect of surface area in the present analysis is variable (i.e., not proportional) with the major impact on drugs that are more poorly absorbed. The V_c values were estimated using the following equation (Rowland and Tozer, 1995):

$$V_c = V_{pl} + \sum_{i=1}^{n} K_{pl:Ti} V_{ti} \quad (21)$$

where $n = 1, 2, 3, 4\ldots$ for blood and well perfused organs, such as plasma, red blood cells, heart, kidney, lung, spleen, liver, and muscles. The V_{pl} and V_t were the plasma volume and tissue volumes, and $K_{pl:T}$ was the plasma-tissue partition coefficient estimated following Poulin's method (Poulin et al., 2001) and based on reported octanol-water partition coefficients of the test compounds in the literature:

$$K_{pl:T} = \frac{K_{vo:w}(V_{np} + 0.3V_{php}) + V_{wp} + 0.7V_{php}}{K_{vo:w}(V_{nT} + 0.3V_{phT}) + V_{wT} + 0.7V_{phT}} \quad (22)$$

where $K_{vo:w}$ was vegetable oil-water partition coefficient; V_{nT}, V_{phT}, and V_{wT} were the fractional contents of neutral lipids, phospholipids, and water in tissue, respectively; and V_{np}, V_{php}, and V_{wp} were the fractional contents of neutral lipids, phospholipids, and water in plasma, respectively. The volumes of well perfused tissues and V_n, V_{ph}, and V_w in plasma and the tissues were obtained from the literature (Poulin and Theil, 2002) and are listed in Table 1. $K_{vo:w}$ was converted from octanol-water partition coefficient (Leo et al., 1971). The average human body weight was assumed to be 70 kg.

The k_s value was set to 0.0315/min assuming that the gastric emptying half-life is one-third of gastric emptying, ~65 min in man (Davenport, 1982). The k_i value was set as 5.025×10^{-3} min^{-1} as the inverse value of the average transit time in human small intestine, ~199 min (Yu et al., 2000).

Among the 51 test compounds, 18 drugs were selected for concentration simulations based on the availability of literature-reported plasma concentration-time profiles in healthy volunteers after a single oral administration under fasting conditions. The values of F_{FP} and V_d in eq. 4 were determined as below:

$$F_{FP} = F_{oral}/F_a \quad (23)$$

and

$$V_d = D_{oral} \times F_{oral}/C_{max} = D_{oral} \times F_{FP} \times F_a/C_{max} \quad (24)$$

where C_{max} was the measured peak concentration after an oral dose. By combining eqs. 4 and 24, plasma concentration profiles were simulated using the following equation where k_{el} was calculated from observed concentration data in humans:

$$C_{pl} = \frac{AC_{max}}{F_a}(Be^{-kst} - Ce^{-(ki+ka,eq)t} - De^{-kelt}) \quad (25)$$

Statistical Analysis. To examine the error associated with the model, a residual (predicted F_a − experimental F_a) plot, error distribution, and assessment of independence test were conducted. When the residuals were normally and independently distributed random effects, the model would be considered adequate, and the assumptions of the model were met. To examine whether the model was suitable in producing relevant F_a data, linear regression analysis of the predicted and experimental F_a was conducted.

Results

Prediction of k_a and F_a. Predicted k_a and F_a values from Caco-2 permeability are listed in Table 2. For most compounds, the predicted $k_{a,eq}$ values ranged from 0.1 to 0.005 min^{-1} with an average of 0.064 min^{-1} and were within the range of commonly observed values (~0.01–0.07 min^{-1}) in humans (Rowland and Tozer, 1995). However, for a few poorly absorbed drugs, such as chlorothiazide, doxorubicin, and saquinavir, the $k_{a,eq}$ values were ~0.0005 min^{-1}, much smaller than the observed values. Predicted F_a values were similar to the experimental values for both extensively and poorly absorbed drugs. The linear regression of predicted versus experimental F_a values (Fig. 2) were highly correlated ($r^2 = 0.934$), and the predicted F_a values for 49 of 51 compounds fell within the 90% confidence intervals. The residuals between predicted and experimental F_a were found to be normally and independently distributed around 1.8 ± 1.1% with approximately equal variance and ranging from −17 to 22% (Fig. 3). The residual test results demonstrate that the model is adequate for prediction of F_a and that the assumptions of the model were met. The predicted V_c values were, in general, much smaller than reported steady-state distribution volumes (Gilman et al., 1990) and close to the physiological volume of well perfused tissues (~32 liters), indicating that the V_c prediction was reasonable.

Concentration Simulation. The simulation parameters and results are presented in Table 3 and Fig. 4, respectively. For 17 of 18 compounds, the simulated kinetic profiles matched their corresponding experimental ones well, indicating that the model is suitable for describing the kinetics of drug absorption and disposition in humans. There was a paucity of data points in the absorptive phase of many of the plasma concentration versus time profiles used in this study. Although not ideal, the impact of this situation is less dramatic than one would expect since absorption occurs after the peak concentrations are observed (i.e., since each point on the curve reflects the rate of absorption and elimination of drug). Therefore, absorption is reflected through many of the time points on the curve. The simulated concentration-time profile for saquinavir was less successful than for the others. Although the predicted AUC of saquinavir, as visualized by the concentration curve, was close to the experimental value, the shapes of the predicted and observed profiles matched poorly. A close correlation between $k_{a,eq}$ and observed k_a values generally led to simulated profiles that matched the clinical result. However, $k_{a,eq}$ (0.0005 min^{-1}) for saquinavir was significantly different from the observed k_a (~0.1 min^{-1}). These data indicate that $k_{a,eq}$ is a key determinant for F_a and can be used as an approximation for predicting plasma concentration profiles. The usefulness of k_a under sink condi-

TABLE 1
The volumes and the fractional volume of well perfused organs in humans
The data were taken from Poulin and Theil, 2002.

	Heart	Kidney	Liver	Lung	Spleen	Muscle	Plasma	Red Blood Cells
Volume (liter)	0.329	0.308	1.82	0.532	0.182	28.3	2.968	2.43
V_w	0.758	0.783	0.751	0.811	0.788	0.76	0.945	
V_n	0.0115	0.0207	0.0348	0.003	0.0201	0.0238	0.0035	
V_{ph}	0.0166	0.0162	0.0252	0.009	0.0198	0.0072	0.00225	

TABLE 2
Predicted values of $k_{a,eq}$, F_a, and V_c for the test compounds, and the comparison between experimental and predicted F_a

Compound	log $K_{oc:w}$[a]	Exp. P_m[b]	Exp. F_a[c]	Pred. V_c	Pred. $K_{a,eq}$	Pred. F_a	Residual[e]
		$\times 10^{-6}$ cm/s	%	l	min^{-1}		
Acebutolol	−0.09	4.50	80	33.7	0.0160	76.1	3.87
Acetyl salicylic acid	−2.25	30.7	100	30.9	0.1192	96.0	4.05
Acyclovir	−1.56	1.16	30	30.9	0.0045	47.3	−17.3
Alprenolol	1.38	25.3	93	32.1	0.0945	95.0	−1.95
Aminopyrine	0.63	36.5	100	31.1	0.1410	96.6	3.44
Atenolol	−1.29	2.70	56	30.9	0.0105	67.6	−11.6
Caffeine	−0.07	50.5	100	30.9	0.1960	97.5	2.50
Chlorpromazine	1.86	19.9	90	35.0	0.0683	93.1	−3.15
Chlorothiazide	−1.15	0.150	10	30.9	0.0006	10.4	−0.39
Cimetidine	0.49	3.06	84	31.0	0.0118	70.2	13.8
Clonidine	0.78	30.1	95	31.2	0.1159	95.8	−0.85
Corticosterone	1.78	21.2	100	34.2	0.0743	93.7	6.33
Desipramine	1.57	21.6	95	32.9	0.0789	94.0	0.99
Dexamethasone	2.16	23.4	92	39.3	0.0714	93.4	−1.42
Diazepam	2.99	71.0	100	82.1	0.1037	95.4	4.62
Diltiazem	2.84	29.8	99	69.5	0.0514	91.1	7.90
Doxorubicin	1.27	0.160	12	31.8	0.0006	10.7	1.28
Furosemide	2.03	5.60	61	37.1	0.0181	78.3	−17.3
Ganciclovir	−2.07	0.380	9	30.9	0.0015	22.7	−13.7
Glycine	−3.21	80.0	100	30.9	0.3108	98.4	1.59
Hydrocortisone	1.61	14.0	89	33.1	0.0508	91.0	−2.00
Hydrochlorothiazide	−0.07	4.60	90	30.9	0.0179	78.0	12.0
Ibuprofen	3.97	52.5	100	175	0.0359	87.7	12.3
Imipramine	4.8	14.1	68	201	0.0084	62.7	5.35
Indomethacin	1	20.4	100	31.4	0.0781	94.0	6.05
Labetalol	1.24	9.31	90	31.8	0.0352	87.5	2.50
Mannitol	−3.100	0.380	18	30.9	0.0015	22.7	−4.71
Meloxicam	0.03	19.5	90	30.9	0.0757	93.8	−3.77
Methotrexate	−1.463	1.20	70	30.9	0.0047	48.1	21.9
Metoprolol	0.51	27.0	95	31.0	0.1044	95.4	−0.41
Naproxen	3.18	54.2	99	101	0.0644	92.8	6.24
Nevirapine	1.81	30.1	90	34.5	0.1048	95.4	−5.42
Nicotine	1.17	19.4	100	31.6	0.0736	93.6	6.39
Phenytoin	2.47	26.7	98	48.4	0.0662	92.9	5.06
Pindolol	0.19	16.7	95	30.9	0.0648	92.8	2.20
Piroxicam	−0.07	35.6	100	30.9	0.1382	96.5	3.51
Propranolol	1.93	27.5	90	35.7	0.0924	94.8	−4.84
Quinidine	3.44	20.4	80	129	0.0189	79.0	0.98
Ranitidine	−0.12	3.40	64	30.9	0.0132	72.4	−8.42
Salicylic acid	−1.44	22.0	100	30.9	0.0855	94.4	5.55
Saquinavir	4.51	0.800	12[d]	196	0.0005	8.9	3.14
Scopolamine	0.21	11.8	100	31.0	0.0457	90.1	9.90
Sulfasalazine	−0.42	0.300	13	30.9	0.0012	18.8	−5.82
Telmisartan	2.41	15.1	90	46.2	0.0393	88.7	1.35
Terbutaline	0.9	1.40	62	31.3	0.0054	51.7	10.3
Testosterone	3.13	72.3	100	95.8	0.0906	94.7	5.26
Timolol	0.03	12.8	95	30.9	0.0497	90.8	4.19
Valproic acid	0.143	48.0	100	30.9	0.1862	97.4	2.63
Verapmil	3.97	69.4	95	175.8	0.0474	90.4	4.59
Warfarin	1.22	38.3	98	31.7	0.1449	96.6	1.35
Zidovudine	−0.58	6.93	100	30.9	0.0269	84.3	15.7

[a] From references: Shah et al., 1989; Glynn and Yazdanian, 1998; Yazdanian et al., 1998; Yazdanian, 1999; Murakami et al., 2000; Kulkarni et al., 2002; Poulin and Theil, 2002.
[b] From references: Collett et al., 1996; Yee, 1997; Caldwell et al., 1998; Pade and Stavchansky, 1998; Yazdanian et al., 1998; Eagling et al., 1999; Hilgendorf et al., 2000; Markowska et al., 2001; Kulkarni et al., 2002; Mandagere et al., 2002.
[c] From references: Benet et al., 1984; Yee, 1997; Pade and Stavchansky, 1998;Yazdanian et al., 1998; Zhao et al., 2001; Kulkarni et al., 2002.
[d] Estimated from animal data (Sinko et al., 2004).
[e] The difference between Exp. F_a and Pred. F_a.

tions in concentration simulation will be discussed in the next section.

Discussion

A novel absorption-disposition kinetic model that links traditional pharmacokinetic and mass transport models was developed in the current study. k_a, $k_{a,eq}$, and F_a were deduced from plasma pharmacokinetics using this model, and the analytical relationships between P_m and k_a and between $k_{a,eq}$ and F_a were found.

Among the 51 structurally diverse compounds used in the evaluation of the model, many are substrates for one or more intestinal drug transporters and some are involved in transcellular and/or paracellular passive diffusion as well. Regardless of the mechanisms of drug absorption from the GI tract, the model produced reasonable estimates of F_a from Caco-2 permeability. The statistical analysis and excellent correlation between experimental and predicted F_a values suggest that the assumptions of the model were met and that the model is adequate for predicting F_a. However, inconsistencies between in vitro and in vivo systems could cause deviations in the prediction of F_a. For example, although Caco-2 cells possess many similarities to enterocytes, their

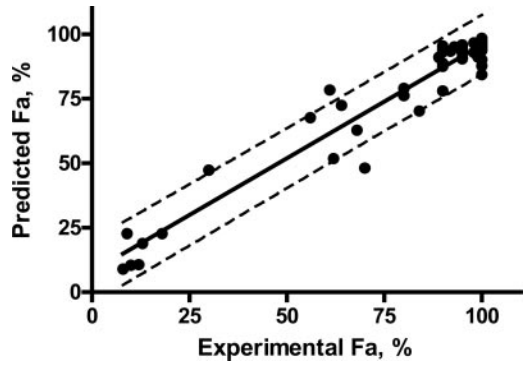

Fig. 2. Linear correlation between predicted F_a and experimental F_a. Pred. F_a = 0.884 (±0.034) * Exp. F_a + 7.41 (±2.87), r^2 = 0.934, Sy = 6.86, F = 672, df = 49, $SS_{reg} = 3.25 \times 10^4$, $SS_{resid} = 2.30 \times 10^3$. Dotted lines represent 95% confidence interval.

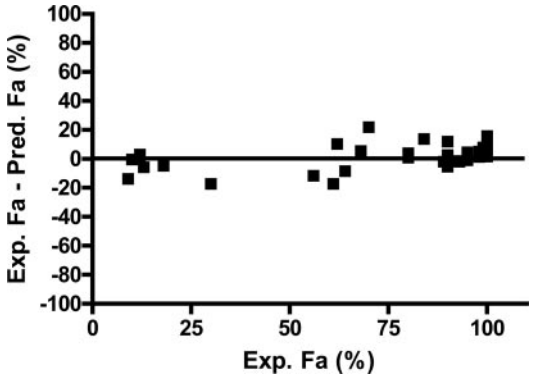

Fig. 3. Residuals between experimental F_a and predicted F_a.

intercellular structures are much tighter than those of enterocytes. Hence, the permeability of paracellular transported drugs, such as cimetidine, atenolol, ranitidine, mannitol, hydrochlorothiazide, and furosemide, could be underestimated in Caco-2 cells (Collett et al., 1996; Hilgendorf et al., 2000). In this study, it was found that Caco-2 permeability results that had transepithelial electrical resistance values close to intestinal tissue allowed for better F_a prediction. The predicted F_a for methotrexate deviated the most from its experimental value (48 versus 70%). Methotrexate is reported to be actively cotransported with H^+ in the small intestine (Sosogi et al., 2003). The underestimated F_a for methotrexate could be due to an underestimated P_m from an experimental system that lacked a physiological pH gradient, or it could be due to poor expression/function of the intestinal transporters involved in methotrexate transport.

In this study, k_a was found to have equilibrium and nonequilibrium analytical solutions. It was observed that $k_{a,eq}$ was a good predictor of plasma concentration profiles for nearly all drugs except for saquinavir. This aberrant result can be explained by the equilibrium and nonequilibrium solutions of k_a. From eq. 14, it is evident that k_a is dependent on the ratio of C_i and C_{pl}. At equilibrium, drug absorption becomes insignificant due to the disappearance of the concentration gradient across the cell monolayer. Therefore, the equilibrium solution of k_a is likely to be related to F_a. On the other hand, drug absorption initially occurs under sink conditions. Therefore, it is possible that, under sink conditions, k_a governs the pharmacokinetic profile during the absorptive phase. Another possible reason for the unsuccessful simulation results of saquinavir in humans could be due to the simplified disposition model that was used. The current absorption-disposition model includes only one disposition compartment. Although the one-compartment disposition worked well for most compounds in the current study, it may result in an inaccurate prediction when a drug has a profile with a significant biphasic decline, such as saquinavir. When using the k_a under sink conditions and with a modified absorption-disposition model (the original model plus a peripheral disposition compartment), we successfully predicted saquinavir plasma concentration profiles in rats (unpublished data). Our findings demonstrate that P_m is a major determinant of k_a, $k_{a,eq}$ is a major determinant of F_a, and the absorption-disposition kinetic model is adequate for describing pharmacokinetic profiles in vivo.

The fundamental difference between the current model and many transport models (Dressman et al., 1984; Amidon et al., 1988; Sinko et al., 1991; Yu and Amidon, 1999) is that the prediction of k_a and F_a is based on drug appearance kinetics in the plasma compartment rather than the drug disappearance kinetics from the intestinal lumen. In the

TABLE 3
Parameters that were used in the plasma concentration simulations, and the source of the experimental data

	A	B	C	D	k_{el}	Clinical Data Source
					min^{-1}	
Atenolol	−1.97	−0.357	−0.780	0.423	0.00206	Sabanathan et al., 1987
Caffeine	0.186	−6.81	−0.988	−5.82	0.00270	Blanchard and Sawers, 1983
Chlorpromazine	0.671	−2.40	−0.947	−1.46	0.000951	Dahl and Strandjord, 1977
Clonidine	0.352	−3.82	−0.968	−2.86	0.00117	Lowenthal et al., 1988
Desipramine	0.601	−2.56	−0.948	−1.61	0.000697	Rudorfer et al., 1984
Diltiazem	1.26	−1.84	−0.973	−0.870	0.00361	Hermann et al., 1983
Hydrochlorothiazide	−3.67	−0.590	−0.823	0.233	0.00117	Sabanathan et al., 1987
Ibuprofen	0.107	−12.2	−1.00	−11.2	0.00520	Lee et al., 1985; Oberbauer et al., 1993
Imipramine	−1.75	−0.274	−0.662	0.388	0.000719	Ciraulo et al., 1988
Indomethacin	0.542	−3.17	−0.997	−2.17	0.00479	Oberbauer et al., 1993
Metoprolol	0.406	−3.64	−0.980	−2.66	0.00291	Toon et al., 1988
Propranolol	0.478	−3.29	−0.983	−2.310	0.00344	Garceau et al., 1978
Quinidine	1.46	−1.65	−0.947	−0.699	0.00231	Mason et al., 1976
Saquinavir	−1.21	−0.0200	0.329	−0.349	0.00566	Roche Laboratories, Nutley, NJ, product information for Fortovase (saquinavir), 2001
Terbutaline	−1.49	−0.195	−0.833	0.638	0.00394	Borgstrom et al., 1989
Timolol	1.36	−1.76	−0.965	−0.793	0.00324	McGourty et al., 1985
Verapamil	1.46	−1.91	−1.03	−0.881	0.00633	McTavish and Sorkin, 1989
Warfarin	0.266	−4.65	−0.969	−3.69	0.000347	Holford, 1986

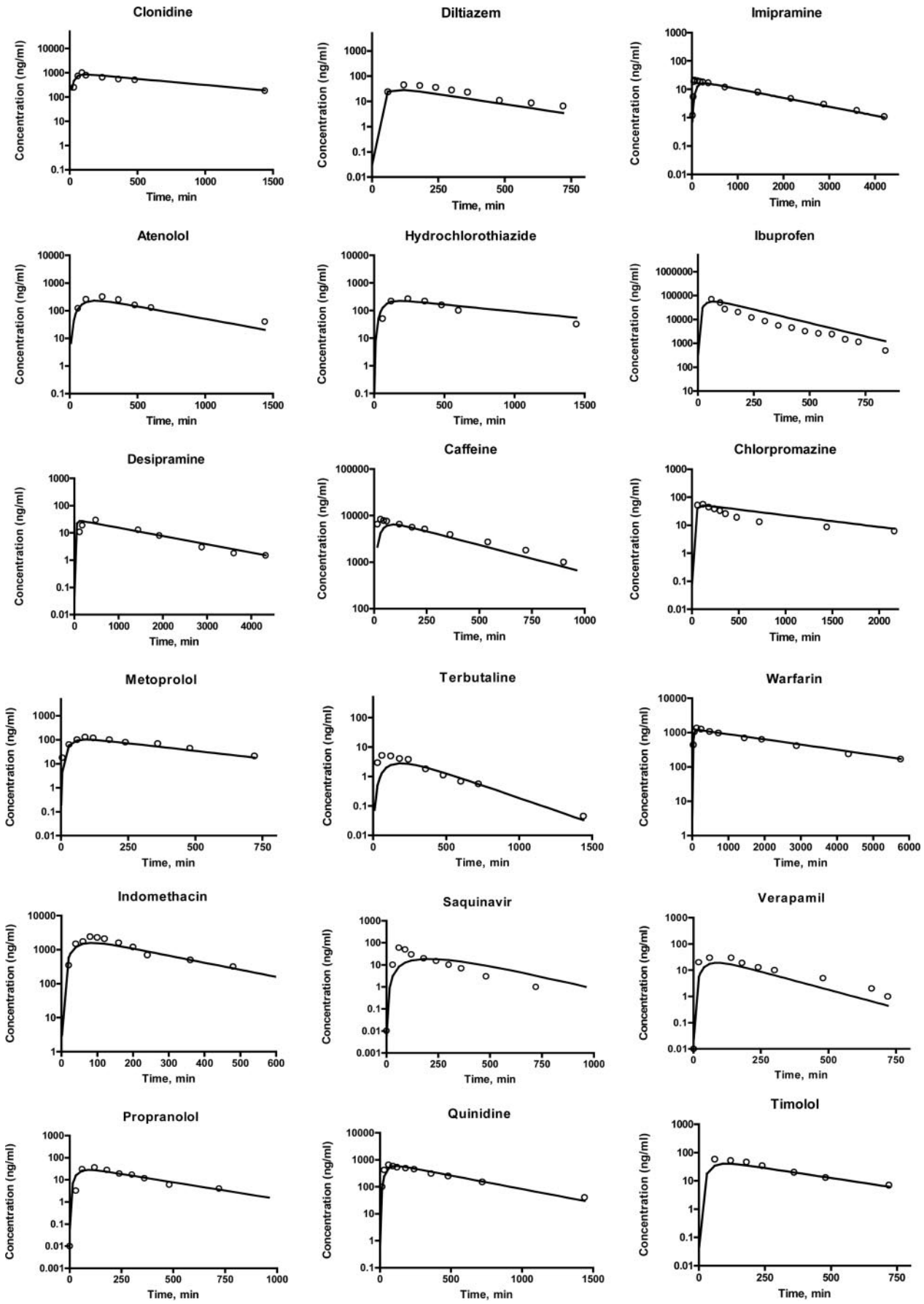

Fig. 4. Plasma concentration-time profiles of selected drugs in humans following a single oral administration. Simulated with $k_{a,eq}$, line and measured, dots.

current model, the absorption rate constant in plasma (k_a) is predicted directly without assuming that the rate of drug appearance in plasma is equal to the drug disappearance rate from the intestinal lumen. This is important since the number of drugs that are known to undergo intestinal and enterohepatic cycling and intestinal accumulation is increasing. This, in turn, leads to a discrepancy in permeability measurements depending on the experimental format and conditions that are used. Although the analytical solution of k_d from the previous transport models has a similar form ($k_d = P_{eff} S/V_i$) to that of k_a ($k_a = P_m S/V_c$) in the current model, they are significantly different in meaning. For example, P_{eff} (effective permeability) is determined from drug disappearance/uptake kinetics in the intestinal lumen, whereas P_m (transcellular permeability) is determined based on the amount of drug that penetrates a tissue barrier (e.g., Caco-2 cells) and appears on the basolateral side. Due to the possible retention of drug molecules that bind to intestinal contents, mucus, and in the intestinal tissue, P_{eff} can also be overestimated. In addition, V_i and V_c can be significantly different. The physiological fluid volume of human intestine (V_i) is ~375 ml (Davenport, 1982), which is much smaller than the physiological volumes of blood and well perfused organs or the volume of distribution in pharmacokinetic definition ($V_c > 30$ liters in general). These discrepancies can lead to differences in the k_a and k_d values. More importantly, since k_d is measured under sink conditions, it is more likely to be similar to k_a under sink conditions, but it can be much greater than $k_{a,eq}$ for some poorly absorbed drugs. Consequently, F_a estimates from k_d [$F_a = k_d/(k_i + k_d)$] (Dressman et al., 1984) are likely to be overestimated, which perhaps explains why many predictive models work well for highly absorbed drugs but work inaccurately for poorly absorbed drugs.

Traditional pharmacokinetic models are based on drug appearance in the blood rather than drug disappearance from the intestinal lumen. The most commonly used oral pharmacokinetic model is a one-compartment extravascular model with k_a as first-order input (absorption) rate constant and k_{el} as the first-order output (elimination) rate constant ($dM_{pl}/dt = k_a M_i - k_{el} M_{pl}$) (Wagner, 1993). The current model differs from traditional pharmacokinetic models in that it links the traditional model to the gastric and intestinal compartments so that the impact of drug kinetics at the absorption site is accounted for. Some of these transport models, such as the CAT model (Yu and Amidon, 1999), also link the intestinal compartments to one or two plasma compartments. However, because k_a is estimated from k_d, the linkage between intestinal compartments and plasma compartments is generally based on simulations rather than a mechanistic link. In contrast, the current model builds the link based on kinetic mechanisms not empirical or simulation approaches. One of the advantages of this model is that by adding these two GI compartments, the initial drug concentration in the intestinal lumen is no longer assumed as a bolus dose but is treated more closely to the in vivo situation after oral administration. The predictive performance of the current model reveals that two GI compartments are adequate for describing the pharmacokinetic profile in plasma. When the gastric emptying and intestinal transit time are modified by a specific drug delivery system, the resulting plasma concentration profiles can be easily predicted by this model. Another advantage is that by adding the GI compartments, F_{FP} (the fraction that survives first-pass metabolism) becomes a determinant for plasma concentrations (eq. 4) rather than F_{oral} as in traditional PK models. This is an advantage since F_{oral} can only be determined by comparing intravenous and oral kinetics, whereas F_{FP} can be directly determined from in vitro metabolism studies such as intestinal and hepatic microsomes.

Drug dissolution rate is considered to be a major determinant of oral drug absorption (Pade and Stavchansky, 1998; Lobenberg and Amidon, 2000). However, in the current study, drug dissolution was excluded from the prediction of F_a and k_a with little impact on the oral absorption estimates. This is likely due to the fact that the dissolution rate of many pharmaceutical compounds is faster than drug permeability through the intestinal wall (Pade and Stavchansky, 1998). Therefore, drug absorption is, in general, controlled by permeability through the intestinal wall rather than by dissolution. When drug dissolution does become the rate-limiting step (i.e., slower than permeability), such as with controlled-release formulations, oral absorption will be controlled by drug dissolution and/or release rate. In this case, the drug dissolution/release rate should be used to replace drug permeability in the F_a and k_a prediction.

The results of this study demonstrate that the current absorption-disposition kinetic model is suitable for describing pharmacokinetics after oral administration. The analytical solutions of k_a allow for the prediction for F_a and oral kinetics from Caco-2 permeability data. It was found that $k_{a,eq}$ is a key determinant of F_a, whereas k_a is likely to be a determinant of absorption kinetic profiles.

In conclusion, a novel absorption-disposition kinetic model that links traditional pharmacokinetic and transport models was developed. The analytical solutions between P_m and k_a and between $k_{a,eq}$ and F_a were found for the first time. The prediction of F_a and plasma concentrations indicates that this model is adequate for quantitative determination of oral kinetics from in vitro permeability. This model also provides a basis for the correlation of in vitro permeability and in vivo absorption kinetics.

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