Human Drug Absorption Kinetics and Comparison to Caco-2 Monolayer Permeabilities

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Purpose. This study aims to assess the drug absorption kinetics of three drugs and compare their resulting first-order intestinal permeation rate constants to their Caco-2 monolayer permeabilities.

Methods. *In vitro* dissolution—*in vivo* absorption analysis was conducted on four formulations of each ranitidine HCl, metoprolol tartrate, and piroxicam to yield apparent and "true" human clinical permeation rate constants. Drug permeability coefficients through Caco-2 monolayers were also determined.

Results. *In vitro* dissolution—*in vivo* absorption analysis revealed different relative and absolute contributions of dissolution and intestinal permeation to overall drug absorption kinetics for various drug formulations and yielded estimates of each drug's true and apparent human intestinal permeation rate constant [k_p = 0.225 hr^{-1}, 0.609 hr^{-1}, and 9.00 hr^{-1} for ranitidine, metoprolol, and piroxicam, respectively]. A rank order relationship was observed for both the apparent and true permeation rate constant with Caco-2 monolayer permeability. The decrease in the true permeation rate constant relative to the apparent permeation rate constant was most significant (almost three-fold) for the least permeable compound, ranitidine.

Conclusions. There were marked differences in the permeation kinetics of ranitidine, metoprolol, and piroxicam. The possibility of an association between absorption kinetics from dosage forms in humans and Caco-2 monolayer permeability may allow for a direct kinetic interpretation of human oral absorption from Caco-2 monolayer permeability values.

KEY WORDS: permeability; oral absorption; Caco-2 cells; pharmacokinetics; human.

INTRODUCTION

We have previously characterized the drug absorption kinetics of metoprolol tartrate from several immediate release tablets through the examination of *in vitro* dissolution–*in vivo* absorption relationships (1). The relative and absolute contributions of dissolution and intestinal permeation kinetics were delineated and yielded an apparent first-order intestinal permeation rate constant. One objective of this work is to extend this analysis to ranitidine and piroxicam, as well as determine a "true" intestinal permeation rate constant for each drug.

A second objective is to compare the intestinal permeation rate constants of the three drugs to their Caco-2 monolayer permeabilities. Caco-2 monolayers are a model to assess oral drug permeability (2–5). Efforts to quantitatively link drug permeability through Caco-2 monolayers to human drug absorption are exemplified by the work of Artursson and Karlsson (6), who reported a sigmoid relationship between the fraction dose absorbed in humans and apparent drug permeability across Caco-2 monolayers. The association between fraction dose absorbed and Caco-2 monolayer permeability has significant practical value. However, a link between Caco-2 monolayer permeability and human drug intestinal permeation is more fundamental. Unlike a Caco-2 monolayer permeability coefficient, fraction dose absorbed is not a kinetic parameter, but a terminal parameter reflecting underlying kinetic events. Hence, a comparison of Caco-2 monolayer permeabilities to human clinical permeation rate constants was investigated.

THEORETICAL

A pharmacokinetics first-order mass transfer coefficient approach was taken to parameterize human drug permeation kinetics. Using this approach to characterize permeation (or overall absorption) kinetics is not without difficulty.[3] Apart from its model-dependent nature and the frequent difficulty in obtaining reliable numerical values from regression of plasma data, numerical values of absorption rate constants for drugs with incomplete absorption has been shown to be potentially overestimated (7,8). With one or more parallel first-order processes competing with absorption (e.g. drug degradation), Notari et al. (7) indicated that the "true" absorption rate constant, denoted here as k_a, is

$$k_a = f_a \cdot k_a^{app} \tag{1}$$

where f_a is the fraction of the dose absorbed at $t = \infty$ and k_a^{app} is the apparent absorption rate constant. Perrier and Gibaldi (8) extended the work of Notari et al. by considering parallel loss of drug after a lag time and truncated absorption.

An expression for a correction factor, Φ, is derived below for the case of truncated absorption due to relatively poor permeability (i.e. "all or none" phenomena of ref 8) where

$$k_p = \Phi \cdot k_p^{app}. \tag{2}$$

k_p is the "true" permeation rate constant. k_p^{app} is the apparent permeation rate constant. It would be expected that $0 < \Phi < 1.0$.

For the case of first-order dissolution and subsequent first-order permeation (9),

$$M_b = M_0\left(1 - \frac{k_p}{k_p - k_d} e^{-k_d t} + \frac{k_d}{k_p - k_d} e^{-k_p t}\right) \tag{3}$$

where M_b is the cumulative mass of drug absorbed into the body, M_0 is the drug dose, and k_d is the first-order dissolution rate constant. Since a permeation model with a terminally truncated permeation window is sought, eq 3 is relevant only for $t \leq t_{win}$ where t_{win} is the time after which permeation ceases (i.e. length of time for intestinal drug permeation). For permeation rate-limited absorption (i.e. $k_p << k_d$), eq 3 yields

$$M_b = M_0(1 - e^{-k_p t}) \tag{4}$$

Equation 4 was derived from the point of view of drug release

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[3] A distinction between permeation and absorption is intended here. Permeation is the process of drug transversing the lumenal absorptive surface. Absorption is the process of both drug dissolution and drug permeation.

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from the dosage form and subsequent loss to (i.e. permeation into) the body for $t \leq t_{win}$; hence, it contains the "true" permeation rate constant, k_p.

Unlike k_p, k_p^{app} is observed from the point of view of drug gain into the body from the intestinal lumen (e.g. observed drug concentration in plasma). Again assuming permeation-rate limited absorption and truncated permeation,

$$M_b = M_b^{t=t_{win}}(1 - e^{-k_p^{app}t}) \quad (5)$$

for all $t \geq 0$ where $M_b^{t=t_{win}}$ is the cumulative mass of drug permeated into the body at $t = t_{win}$. As noted by Perrier and Gibaldi (8) and Leeson and Weintraub (10), the idealized relationship between M_b and t in eq 5 is not log-linear for $f_a < 1$, but may often be fitted to eq 5 to arrive at k_p^{app}.

Equating eqs (4) and (5),

$$M_0(1 - e^{-k_p t}) = M_b^{t=t_{win}}(1 - e^{-k_p^{app}t}) \quad (6)$$

for $t \leq t_{win}$. Selecting $t = t_{win}$, substituting in eq 2, and simplifying gives

$$e^{-\Phi k_p^{app} t_{win}} - f_a e^{-k_p^{app} t_{win}} = 1 - f_a \quad (7)$$

Hence, given f_a, k_p^{app} and t_{win}, the correction factor Φ can be determined from eq 7 and then applied in eq 2 to yield k_p.

MATERIALS AND METHODS

Formulations and Clinical Studies

Four formulations of each ranitidine hydrochloride, piroxicam, and metoprolol tartrate were studied. For each drug, a fast, medium, and slow dissolving "immediate release" formulation was developed at the University of Maryland School of Pharmacy and evaluated in a cross-over bioequivalency study along with the reference product. The analysis of the critical manufacturing variables and clinical results of the 300 mg (ranitidine equivalent) ranitidine hydrochloride tablet (11–13), 20 mg piroxicam capsule (14) and 100 mg metoprolol tartrate tablet (15) have been reported.

Estimation of Apparent Permeation Rate Constants from *In Vitro* Dissolution–*In Vivo* Absorption Relationships

For each drug, the apparent permeation rate constant k_p^{app} was estimated using a previously described approach (9). This approach employed the model:

$$F_a = \frac{1}{f_a}\left(1 - \frac{\alpha}{\alpha - 1}(1 - F_d) + \frac{1}{\alpha - 1}(1 - F_d)^{\alpha}\right) \quad (8)$$

where F_a is the fraction of the total amount of drug absorbed at time t, f_a is the fraction of the dose absorbed at $t = \infty$, α is the ratio of the first-order apparent permeation rate constant to the first-order dissolution rate constant, and F_d is the fraction of the dose dissolved at time t. Assumptions of this model have been described and include first-order dissolution, apparent first-order permeation, the equality of *in vitro* dissolution and in vivo dissolution profiles (i.e. *in vitro* dissolution profile serves as a perfect surrogate for *in vivo* dissolution profile), and no physical or chemical degradation of the drug in the gastrointestinal lumen. Gastric emptying influences are not considered. Previous applications of eq 8 (1,9) refer to a "permeation rate constant k_p". With regard to this work, previous permeation rate constants should be interpreted as apparent permeation rate constants k_p^{app}. Here, k_p is taken as the "true" permeation rate constant.

Using eq 8, "*in vitro* dissolution—*in vivo* absorption correlation" analysis was conducted for ranitidine HCl and piroxicam formulations in a manner similar to that reported for metoprolol tartrate (1). For four individual piroxicam *in vitro-in vivo* trajectories (one for Feldene^R and three for slow), convergence was not attained due to large F_a relative to F_d at 40 min. These four "outliers" are consistent with the observation that dissolution was rate limiting in these formulations (see Results and Discussion).

Estimation of True Permeation Rate Constants

From eq 7, the correction factor Φ was determined for metoprolol and ranitidine and then applied to k_p^{app} using eq 2 to yield k_p. In the determination of Φ, the value of t_{win} was selected as the time at which $F_a \geq 95\%$. Piroxicam's k_p^{app} was not analyzed according to eq 7 since piroxicam absorption was not permeation rate limited (see Results and Discussion), as required by eqs 4 and 5.

Caco-2 Monolayer Permeability Determinations

Caco-2 cells were obtained from ATCC (Rockville, MD) and grown at 37°C in T-75 flasks in an atmosphere of 5% CO_2 and 95% RH using Delbecco's Modified Eagles Media (DMEM) supplemented with 10% FBS, 1% NEAA, and 0.5% penicillin/streptomycin. Cells were passaged at 80–90% confluency using a 0.02% EDTA/0.05% trypsin solution. Media was changed about every 48 hr. Between passage numbers 35–45, cells were seeded on polycarbonate Transwell^R filters (Corning-Costar, Cambridge, MA) (0.4 μm mean pore size; 4.71 cm^2 area) at a density of 4×10^5 cells/cm^2. These cells were cultured for 21–28 days and then used for permeability studies.

Permeability studies were conducted in Hank's balanced salts solution (pH = 6.8) at 37°C and 50 oscillations per min. Monolayer integrity was evaluated by using ^{14}C-mannitol permeability ($<2 \times 10^{-7}$ cm/sec) and transepithelial electrical resistance (TEER) in growth media ($>940\ \Omega \cdot cm^2$ at ambient room temperature after subtracting a "filter" resistance of 750 $\Omega \cdot cm^2$). Donor drug concentrations were 4.275 mM ranitidine HCl, 0.0569 mM piroxicam, and 0.438 mM metoprolol tartrate. Transport studies were conducted in both the apical-to-basolateral and the basolateral-to-apical directions. Drug was quantified using HPLC. In each study, mass balance was determined and ranged from 90 to 101%.

Control transport studies were also conducted across Transwell^R filters without cells to determine P_{filter}. Caco-2 monolayer permeability (P_m) was estimated by correcting the effective permeability (P_{eff}) for P_{filter} according to $P_{eff}^{-1} = P_m^{-1} + P_{filter}^{-1}$.

RESULTS AND DISCUSSION

In Vitro Dissolution–*In Vivo* Absorption Relationships and Apparent Permeation Rate Constants

For ranitidine, drug absorption ceased ($F_a \geq 95\%$) at 2.2 (±0.1) hr. t_{win} was independent of formulation ($p = 0.45$),

suggesting permeation rather than dissolution was the rate limiting step in overall absorption. For piroxicam, drug absorption was completed at generally longer times for more slowly dissolving formulations (p = 0.03), suggesting dissolution-rate limited absorption for at least the slower dissolving formulations. For fast, medium, Feldene^R, and slow, drug absorption was complete at 1.1 (±0.1) hr, 1.6 (±0.2) hr, 1.4 (±0.1) hr, and 1.8 (±0.1) hr, respectively. For metoprolol, t_{win} was 2.2 (±0.2) hr. Like ranitidine, t_{win} was independent of formulation (p = 0.33), suggesting permeation rate-limited absorption from all four of the formulations.

Figure 1a and 1b illustrate the fraction absorbed versus fraction dissolved plots for the ranitidine and piroxicam formulations, respectively. The plot for the metoprolol formulations has been previously shown (1). Such a plot for each formulation can be interpreted to be the formulation's trajectory in the *in vitro* dissolution–*in vivo* absorption phase plane and can be analyzed to reveal the relative contributions of dissolution and intestinal permeation to overall drug absorption kinetics. The qualitative differences in the "*in vitro–in vivo*" trajectories for ranitidine and piroxicam highlights the two differnt rate-limiting phenomena for the two drug sets, as described below.

Ranitidine's mean values of α are listed in Table Ia and were 0.0646, 0.0943, 0.0964, and 0.156 for fast, Zantac^R, medium, and slow, respectively, indicating increasingly greater dissolution rate-limited absorption for progressively slower dissolving formulations. However, since $\alpha << 1$ in all cases, overall ranitidine absorption was markedly permeation rate-limited, even from slow, yielding a "reverse L" trajectory for each formulation in Fig. 1a. From $k_p^{app} = \alpha\, k_d$, k_p^{app} was estimated and is listed in Table Ia. k_p^{app} was independent of formulation (p = 0.67) and gave a mean value of 0.597 hr^{-1} across all rantidine formulations.

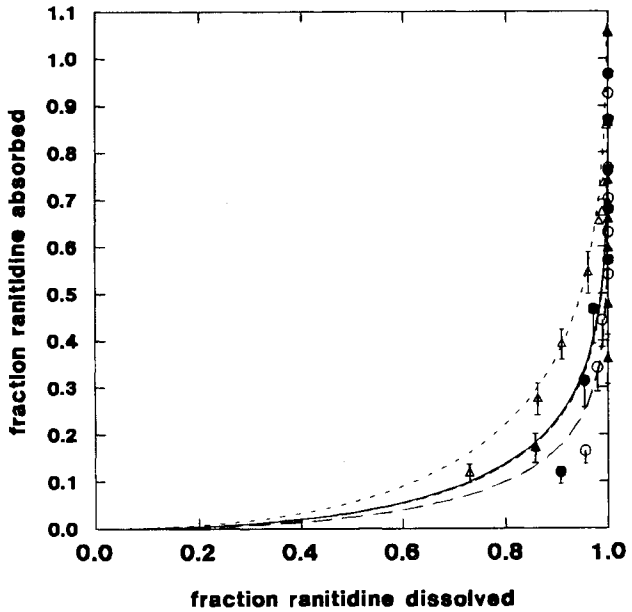

Fig. 1a. Mean F_a vs. F_d trajectory (i.e. *in vitro* dissolution—*in vivo* absorption relationship) of Zantac^R (closed circle) and three test formulations [fast (open circle), medium (closed triangle), and slow (open triangle)]. The curves are the mean fits of eq 8 to each formulation's *in vitro-in vivo* data.

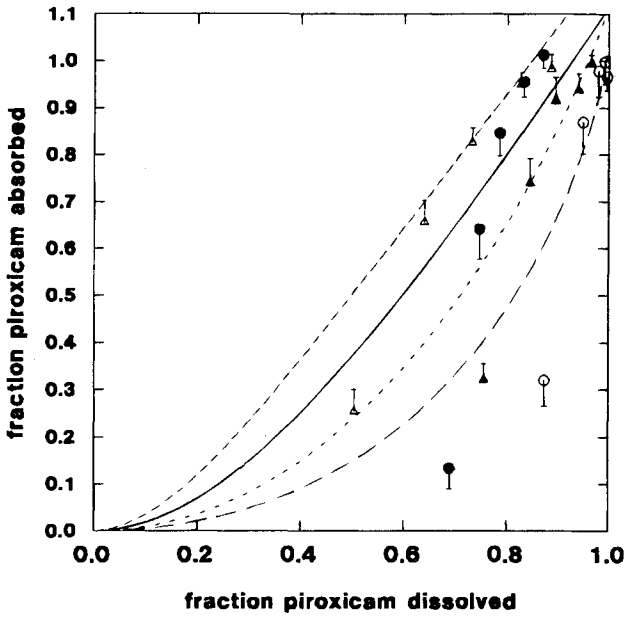

Fig. 1b. Mean F_a vs. F_d trajectory (i.e. *in vitro* dissolution—*in vivo* absorption relationship) of Feldene^R (closed circle) and three test formulations [fast (open circle), medium (closed triangle), and slow (open triangle)] where eq 8 was successfully fit. The curves are the mean fits of eq 8 to each formulations' *in vitro-in vivo* data.

The mean (±SE) F_a versus F_d trajectory for the piroxicam formulations are plotted in Fig 1b. Significant systematic deviations exist between the experimental and fitted data, indicating a deficiency in the eq 8 in characterizing the relationship between dissolution and absorption for piroxicam. In particular, the observed F_d at 20 min (first data point in the trajectory) was too large. Although overall absorption can be expected to be more complex than the model underlying eq 8, the possibility of the observed F_d at 20 min being too large appears real since the dissolution medium was simulated gastric fluid. Unlike more neutral pH conditions such as in the small intestine, this highly acidic compendial dissolution medium provides sink conditions (data not shown). Hence, dissolution may be faster *in vitro* than *in vivo*, at least at 20 min, and thus may account for the poor agreement between the observed and fitted F_a vs. F_d trajectories in Fig 1b.

While recognizing the limitations of eq 8 for these piroxicam formulations, mean values of α are listed in Table Ib. Values of α were 0.896, 1.54, 3.42, and 6.50 for fast, medium, Feldene^R, and slow, respectively, indicating increasingly greater dissolution rate-limited absorption for progressively slower dissolving formulations. From $k_p^{app} = \alpha\, k_d$, k_p^{app} for piroxicam was estimated and is listed in Table Ib. k_p^{app} was independent of formulation (p = 0.43) and gave a mean value of 9.00 hr^{-1} across all piroxicam formulations.

In Table Ic, the mean k_p^{app} for metoprolol was 0.759 hr^{-1} and was independent of formulation (p = 0.09) as previously reported (1).

True Permeation Rate Constants and Their Manifestation in *In Vitro* Dissolution–*In Vivo* Absorption Relationships

Using eq 7 and values for k_p^{app}, t_{win}, and f_a, a value for Φ was determined and then applied to eq 2 to yield a value for

Table Ia. Rate Considerations in Ranitidine HCl Oral Absorption: Absolute and Relative Contributions of Dissolution and Intestinal Permeation to Overall Ranitidine Absorption Kinetics

Formulation	f_a (±SE)	alpha (±SE)	k_d (±SE) (hr^{-1})	k_p^{app} (±SE) (hr^{-1})	t_{win} (±SE) (hr)	Φ (±SE)	k_p (±SE) (hr^{-1})
Fast	0.502 (±0.018)	0.0646 (±0.0095)	10.4 (±1.4)	0.680 (±0.095)	2.00 (±0.17)	0.361 (±0.19)	0.113 (±0.030)
Zantac^R	0.520[a] (±0.016)	0.0943 (±0.0181)	6.18 (±0.30)	0.583 (±0.108)	2.10 (±0.20)	0.399 (±0.10)	0.227 (±0.041)
Medium	0.541 (±0.016)	0.0964 (±0.0194)	5.33 (±0.29)	0.514 (±0.100)	2.50 (±0.34)	0.419 (±0.18)	0.206 (±0.036)
Slow	0.517 (±0.021)	0.156 (±0.020)	3.94 (±0.64)	0.613 (±0.075)	2.14 (±0.16)	0.374 (±0.13)	0.233 (±0.031)
Mean	0.520[a] (±0.009)	—	—	0.597 (±0.047)	2.18 (±0.12)	0.389 (±0.009)	0.225 (±0.017)

[a] Assigned based upon ref (16).

Table Ib. Rate Considerations in Piroxicam Oral Absorption: Absolute and Relative Contributions of Dissolution and Intestinal Permeation to Overall Piroxicam Absorption Kinetics

Formulation	f_a (±SE)	alpha (±SE)	k_d (±SE) (hr^{-1})	k_p^{app} (=k_p) (±SE) (hr^{-1})
Fast	0.949 (±0.018)	0.896 (±0.138)	8.10 (±0.60)	7.26 (±1.12)
Medium	0.893 (±0.020)	1.54 (±0.24)	4.66 (±0.10)	7.17 (±1.10)
Feldene^R	0.896 (±0.019)	3.42 (±0.84)	3.13 (±0.20)	10.7 (±2.6)
Slow	0.819 (±0.022)	6.50 (±2.17)	1.75 (±0.05)	11.3 (±3.8)
Mean	0.892 (± 0.011)	—	—	9.00 (±1.14)

k_p. While k_p^{app} summarizes permeation kinetics from the viewpoint of drug addition to the systemic circulation, k_p is a kinetic parameter for drug loss from the gastrointestinal lumen due to drug permeation. As a parameter characterizing drug propensity to permeate the intestinal membrane, k_p^{app} essentially fails to consider drug that contributes to the transepithelial drug concentration gradient, but does not permeate. Hence, k_p^{app} can be expected to be upwardly biased and increasingly overestimated for drugs with lower f_a.

Φ and k_p values for ranitidine and metoprolol are given in Tables Ia and Ic, respectively. Since only about half the total ranitidine dose was absorbed, but presumably all drug was in solution in the gastrointestinal lumen, Φ for ranitidine was much less than one. Across all formulations, Φ was 0.389 and was perhaps independent of formulation ($p = 0.07$). For metoprolol, which is more completely absorbed than ranitidine, Φ was closer to one (Φ = 0.830) and was independent of formulation ($p = 0.14$). Since piroxicam absorption was not permeation rate-limited, k_p was taken as k_p^{app} (Table Ib).

k_p for piroxicam, metoprolol, and ranitidine were 9.00 (±1.14) hr^{-1}, 0.609 (±0.085) hr^{-1}, 0.225 (±0.017) hr^{-1}, respectively. It should be noted that k_p for piroxicam may be inflated if *in vitro* dissolution was markedly faster than *in vivo* dissolution, as speculated above. Using the small intestinal transit time for dosage forms of 3 (±1) hours as a reference (17), piroxicam's k_p can be considered large. A high k_p for piroxicam is in agreement with piroxicam's rapid and complete

Table Ic. Rate Considerations in Metoprolol Tartrate Oral Absorption: Absolute and Relative Contributions of Dissolution and Intestinal Permeation to Overall Metoprolol Absorption Kinetics

Formulation	f_a (±SE)	alpha (±SE)	k_d (±SE) (hr^{-1})	k_p^{app} (±SE) (hr^{-1})	t_{win} (±SE) (hr)	Φ (±SE)	k_p (±SE) (hr^{-1})
Lopressor^R	0.923 (±0.025)	0.0877 (±0.0328)	9.24 (±0.12)	0.810 (±0.268)	1.89 (±0.16)	0.852 (±0.063)	0.648 (±0.248)
Fast	0.962 (±0.024)	0.0743 (±0.0178)	8.34 (±0.48)	0.619 (±0.139)	2.25 (±0.56)	0.930 (±0.042)	0.591 (±0.138)
Medium	0.882 (±0.034)	0.0995 (±0.0181)	4.02 (±0.17)	0.400 (±0.068)	1.88 (±0.26)	0.846 (±0.045)	0.330 (±0.048)
Slow	0.885 (±0.030)	0.648 (±0.103)	1.63 (±0.11)	1.05 (±0.16)	2.67 (±0.36)	0.736 (±0.066)	0.778 (±0.153)
Mean	0.910 (±0.015)	—	—	0.759 (±0.098)	2.22 (±0.18)	0.830 (±0.031)	0.609 (±0.085)

absorption (18). The manifestation of piroxicam's large k_p is evident in the "hockey stick" and "straight line" relationships between F_a and F_d for the various formulations in Fig. 1b, where permeation is occurring soon after dissolution due to high piroxicam intestinal permeability.

A simple description of the permeation rate kinetics (i.e. high or low) of metoprolol and ranitidine appears more difficult. Since each drug is absorbed in sufficient quantities for clinical effect, even in spite of permeation rate-limited absorption, the absorption rate constant for each metoprolol and ranitidine can be considered sufficiently fast.

Comparison of Caco-2 Monolayer Permeability Coefficients and Human Clinical Permeation Rate Constants

Figure 2 plots the apparent and true permeation rate constants versus Caco-2 monolayer permeability. From left to right, the data are ranitidine [P_m = 4.25 (±0.58) × 10^{-7} cm/sec], metoprolol [P_m = 1.07 (±0.03) × 10^{-5} cm/sec], and piroxicam [P_m = 9.13 (±0.12) × 10^{-5} cm/sec]. A rank order relationship was observed for both apparent and true permeation rate constants with Caco-2 monolayer permeability. More drugs need to be evaluated. Figure 2 also highlights the impact of permeability (or fraction absorbed, f_a) on the degree of difference between k_p^{app} and k_p, where the relative difference between k_p^{app} and k_p is larger for less permeable compounds. For ranitidine, k_p was almost three-fold lower than k_p^{app}.

The relationships between f_a and Caco-2 monolayer permeability, such as that of Artursson and Karlsson, already provide a basis to assess a drug candidate's oral absorption potential. Similarly, a known relationship between k_p and P_m may provide a basis to assess a drug candidate's intestinal permeation kinetics. While it is not clear that a mapped relationship between

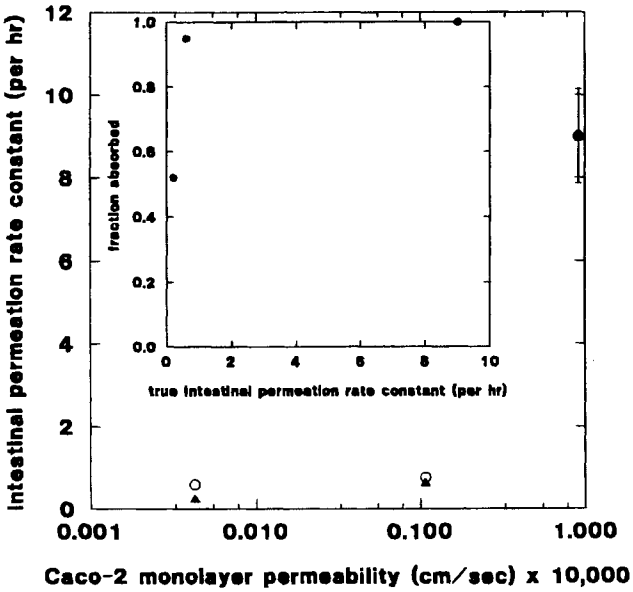

Fig. 2. Plot of apparent (open circle) and true (closed triangle) permeation rate constant versus Caco-2 monolayer permeability of ranitidine (least permeable), metoprolol, and piroxicam (most permeable). A rank order relationship was observed for each permeation rate constant and Caco-2 monolayer permeability. The inset plot shows fraction absorbed versus true permeation rate constant.

k_p and P_m would be generally more useful from a drug screening perspective than a relationship between f_a and P_m, the association between k_p and P_m is more fundamental than the association between f_a and P_m. k_p and P_m are each kinetic parameters; f_a is not a kinetic parameter, but a terminal parameter reflecting underlying kinetic events.

An inset plot of f_a versus k_p is drawn in Fig. 2. While more data are needed, this preliminary observation of f_a versus k_p is similar to other extent absorbed versus permeability profiles. The f_a versus k_p plot in Fig. 2 suggests that drugs can be several fold more permeable than metoprolol in humans (e.g. piroxicam) but will not be substantially more absorbed than metoprolol. Meanwhile, a drug can be just three-fold less permeable than metoprolol in humans (e.g. ranitidine), but will provide for a smaller f_a. These observations based upon k_p agree with Artursson and Karlsson's plot of f_a versus Caco-2 monolayer permeability, the f_a versus human jejunum permeability plot of Faberholm, Johansson, and Lennernas (19), and the f_a versus rat intestine permeability plot of Faberholm, Johansson, and Lennernas (19).

A relevant question is 'How do the observed clinical rate constants for ranitidine, metoprolol, and piroxicam compare to the expected values from their permeability coefficients?' An expected value (20) for a first-order permeation rate constant from a permeability coefficient is

$$k_p = (A/V)P \tag{9}$$

where A is the surface area available for permeation and V is the volume in which drug is dissolved. An estimate of A/V for the human small intestine is 11 cm^{-1}, assuming the small intestine is a simple smooth cylinder of length 282 cm (21), radius 1.5 cm (21), and volume 250 ml (22). Experimentally determined estimates here of A/V (via k_p/P_m) for ranitidine, metoprolol, and piroxicam are 147.1 (±22.9) cm^{-1}, 15.8 (±2.3) cm^{-1} and 27.4 (±3.5) cm^{-1}, respectively, in spite that essentially identical Caco-2 monolayers were used for each drug and the same A and V presumably apply for the human volunteers across the differing drug trials.

Several possible reasons for the differences in A/V (or k_p/P_m) for ranitidine, metoprolol, and piroxicam from 11 cm^{-1} exist. A significant portion of ranitidine's large value of 147.1 cm^{-1} may be attributed to ranitidine's mechanism of permeation and the comparative biology of the tight junctions of Caco-2 monolayers versus the tight junctions of human small intestine. Ranitidine has been shown to permeate Caco-2 monolayers predominately via the tight junctions (23); presumably, this paracellular transport mechanism is also relevant to human intestinal epithelia *in vivo*. However, Caco-2 monolayers are generally recognized as epithelia with "tighter" overall tight junctional structure than human small intestinal epithelia (24,25). Consistent with these observations, the resistance of excised human small intestine is 42.0 (±1.6) $\Omega \cdot cm^2$ at 37°C (26), while Caco-2 monolayer resistance under identical conditions is 334 (±5) $\Omega \cdot cm^2$, a 7.95 (±0.3)-fold difference. Hence, a significant portion of ranitidine's large k_p/P_m value of 147.1 cm^{-1} may be attributed to differences in the structural biology between Caco-2 monolayer tight junctions and human small intestinal tight junctions.

Considering the simplifying approach eq 8 represents, the experimentally determined k_p/P_m values of 15.8 cm^{-1} and 27.4 cm^{-1} for metoprolol and piroxicam are remarkably close to an

idealized value of 11 cm^{-1}. Complexities that could be expected to influence k_p/P_m values include differences in intrinsic permeability between Caco-2 monolayers and small intestinal epithelia, true surface area differences between Caco-2 monolayers and small intestinal epithelia, and a reduced effective surface area for these drugs due to their nearly complete absorption. While metoprolol's k_p/P_m value matches an idealized value, Lennernas *et al.* (27) determined metoprolol's permeability across human proximal jejunum to be 1.5×10^{-4} cm/sec, which is over 10-fold larger than metoprolol's Caco-2 monolayer permeability here. Hence, assuming an idealized 11 cm^{-1} value, intrinsic permeability differences between Caco-2 monolayers and human proximal jejunum appear to be compensated by true and effective surface area differences and/or regional permeability effects.

In conclusion, *in vitro* dissolution—*in vivo* absorption analysis was conducted to yield a "true" human clinical permeation rate constant, which had a rank order relationship with P_m. Because the potential utility of measuring drug permeability across Caco-2 monolayers to predict human absorption kinetics of oral drug candidates, dosage forms of more drugs need to be evaluated in mapping the relationship between k_p and P_m. Such efforts will also contribute toward an understanding of drug absorption kinetics and Caco-2 monolayers as a model for drug absorption.

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