Method to Estimate the Rate and Extent of Intestinal Absorption in Conscious Rats Using an Absorption Probe and Portal Blood Sampling

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Purpose. A variety of methods exist which determine the rate and extent of intestinal absorption. The method described here employs an internal absorption reference probe and portal blood sampling in unanesthetized rat. ***Methods.*** Theophylline and tritiated water were selected as absorption reference probes since they are quantitatively absorbed in conscious rat. The fraction of an intestinal dose which reaches portal blood was determined from the resulting portal-systemic blood concentration gradients of the drug relative to the absorption probe. The absorption probes provide a means to calculate the drug mass reaching portal blood without the need of measuring the portal blood flow rate. The technique was evaluated with verapamil and a well-absorbed 5-lipoxygenase inhibitor, A-79035. ***Results.*** The fraction of an intrajejunal dose of A-79035 reaching the portal vein (F_G) was 0.86 using theophylline as the absorption probe. Verapamil, which is susceptible to extensive hepatic first-pass elimination, was completely absorbed (F_G = 0.98) within 1 hour, but was only 21.4% bioavailable. Absorption rate constants, estimated from initial appearance rates in portal blood, were used to monitor factors that affect drug absorption. For example, with a dose solution containing 30% PEG-400, the absorption rate constants of theophylline and A-79035 were significantly reduced. Anesthesia reduced the absorption rate constant for theophylline in rats by 40% compared to conscious animals. ***Conclusions.*** The technique detailed here allows reliable, direct measurement of intestinal absorption which may assist in characterizing oral dosing for novel therapeutic agents.

KEY WORDS: intestinal drug absorption; conscious rat; portal blood; theophylline; chronic cannulation; verapamil; 5-lipoxygenase inhibitor.

INTRODUCTION

Specific barriers to intestinal drug absorption are not routinely identified by the most commonly used methods to quantitate oral drug delivery. The most prevalent *in vivo* method reported in the literature to estimate the rate and extent of intestinal absorption is measurement of the disappearance of a substance from the perfused intestinal lumen of anesthetized rats (1-3). In many cases this approach is satisfactory, but may overestimate absorption rates for substances that are degraded or metabolized in the intestinal lumen. For example, compounds such as peptides, peptidomimetics and prodrugs, are frequently susceptible to preabsorptive elimination. Bioavailability measurements provide an alternate, overall estimate of the extent to which a substance reaches the general circulation, but includes the contribution of hepatic clearance. The most reliable method to estimate intestinal absorption is measurement of the fraction of a dose entering portal blood. Such a specific assessment of drug delivery from intestinal lumen to portal blood provides a well defined measure of the two primary barriers to intestinal delivery, effects of intestinal epithelial transport and presystemic biotransformation. Through the use of *in vitro* preparations, the contributions of permeation and metabolism can be separated if necessary; but the results of our experience suggest that the combined measurement is an exceptionally utilitarian estimate of intestinal absorption.

In principle, the measurement of an intestinal drug dose appearing in portal blood requires sampling both portal and systemic blood. As intestinal absorption begins, portal concentrations rise with subsequent elevations of systemic concentrations. As absorption terminates, the intestine provides no additional drug and portal levels reflect the systemic concentrations. As a result, the mass of drug measured in portal blood (M_p) is taken as the sum of the contribution of intestinal absorption (M_a) over time, and the mass of drug returned to the portal vein from the systemic circulation (M_s).

$$M_p = M_a + M_s \tag{1}$$

The total mass of drug appearing in the portal vein (M_p) to time t, whether of intestinal or systemic origin, is simply the product of portal blood flow and the area under the portal concentration-time curve ($AUC_{p(0-t)}$). These qualitative relations can be formalized in the following fashion.

$$M_p = Q_p * AUC_{p(0-t)} \tag{2}$$

and

$$M_s = Q_p * AUC_{s(0-t)} \tag{3}$$

Where Q_p is the average portal blood flow rate and $AUC_{s(0-t)}$ is the area under the systemic concentration-time curve to time t. Therefore, the mass of drug in the portal circulation as a result of intestinal absorption is expressed as:

$$\begin{aligned} M_a = M_p - M_s &= Q_p * (AUC_p - AUC_s) \\ &= Q_p * \Delta AUC \end{aligned} \tag{4}$$

where ΔAUC is the difference between the portal and systemic AUC's. Therefore, ΔAUC is independent of distribution, excretion and post-absorptive metabolism. Expressing Eq 4 relative to the dose(D) yields the fraction of dose absorbed from the gut.

$$F_G = M_a/D = Q_p * \Delta AUC/D \tag{5}$$

AUC's can be expressed as a linear trapezoidal summation of the concentration-time curves as follows:

$$AUC = \sum_{i=1}^{i=n} [(C_{i+1} + C_i)/2] * (t_{i+1} - t_i) \tag{6}$$

Consequently, the fraction of drug dose absorbed by the intestine (F_G) can by rearrangement be expressed as a function of the portal-systemic concentration difference or gradient at any moment in time.

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$$F_G = \frac{1}{D} * \sum_{i=1}^{i=n} \{[(C_p - C_s)_{i+1} + (C_p - C_s)_i]/2\} * (t_{i+1} - t_i) * (Q_p^i + Q_p^{i+1})/2 \quad (7)$$

where C_p and C_s are drug concentrations in portal and systemic blood. For practical applications Eq 7 can be used to calculate fraction of drug absorbed at any point in time or for final drug absorption where the relationship reduces to Eq 5.

In small animals, such as the rat, electronic flow probes are available to measure portal blood flow rates. Electronic flow probes can be chronically located on the portal vein of a rat but frequently do not maintain proper orientation and function for accurate measurement of portal blood flow rates over a 2 to 3 week period. Maintaining a colony of chronically cannulated animals requires a substantial investment in electronic flow probes. Therefore, we investigated an alternate approach to estimate the extent of absorption in conscious rats. Coadministration of drug together with a completely absorbed reference probe ($F_G' = 1$) is in principle similar to indicator-dilution techniques (4). By dosing and sampling all solutes at the same sites and times, factors such as blood flow rate and extent of mixing with blood prior to sampling, are circumvented and:

$$F_G/F_{G'} = F_G = \Delta AUC/\Delta AUC_r * D_r/D \quad (8)$$

where ΔAUC refers to the difference between AUC_p and AUC_s for the drug and reference probe (ΔAUC_r), and reflects the net amount of drug reaching the portal blood. This study explores the use of tritiated water and theophylline as probes for estimating the rate and extent of drug absorption in conscious rat.

MATERIALS AND METHODS

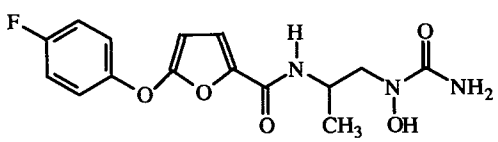
Materials

Tritiated water (1 mCi g^{-1}) was purchased from DuPont-NEN (Boston, MA). Tritiated polyethylene glycol (PEG)-458 (40.2 mCi $mmole^{-1}$) was synthesized by the method of Dellaria, et al. (5). Aminophylline, verapamil, and fluorescein were purchased from the Aldrich Chemical Co. (Milwaukee, WI). Ampicillin was purchased from Sigma Chemical Co. (St. Louis, MO) Nembutal (Abbott) and ketamine were also available commercially. A-79035 was obtained from the Drug Sample Department of Abbott Laboratories. All other reagents were of analytical grade.

Physicochemical Properties

Octanol/water distribution coefficients were determined by the shake-flask (6) method. Briefly, about 0.5 mg of compound was dissolved in 1 ml n-octanol. To a test tube containing 5 ml of aqueous phase was added 0.5 ml of the n-octanol drug solution followed by shaking for 4 hours at room temperature and centrifugation. Samples of each phase were appropriately diluted and assayed by HPLC. After adjusting for the dilution factor, the distribution coefficient was calculated as the peak area ratio of the n-octanol phase and the aqueous phase, expressed as log D.

Osmolarities were determined by freezing point depression using a Model 3W2 osmometer from Advanced Instruments, Inc.



A-79035

Scheme 1.

Surgery

Male Sprague-Dawley rats (200-250 g) were anesthetized with ketamine (20 mg kg^{-1}, i.m.) followed by pentobarbital sodium (10 mg kg^{-1}, i.p.). Catheters were prepared and surgically implanted under aseptic conditions using the procedure of Kimura, et. al (7). Briefly, a 5 cm vertical abdominal midline incision was made through only the skin followed by dissection from the musculature. A small (0.25-cm) skin incision was made behind the neck and, using a crochet hook, four infusion sets were pulled through a subcutaneous tunnel from the neck region to the abdominal skin opening. Following a midline incision through the abdominal wall, the infusion sets were passed into the abdominal cavity through a small puncture in the abdominal wall. The infusion sets were filled with sterile saline containing heparin at 10 units ml^{-1}. The portal vein was exposed by retracting the mesenteric bed onto sterile gauze saturated with sterile saline.

A PE 60 catheter was inserted into the portal vein over a 25-gauge spinal needle just proximal to the liver; the PE 60 was advanced into the portal vein about 1 cm over the needle. The needle was removed leaving the PE 60 catheter in the portal vein. The catheter was secured to abdominal cavity musculature 3 cm from the portal vein entrance with a drop of cyanoacrylate adhesive. The catheter was secured such that it ran parallel to the portal vein. The free end of the PE 60 catheter was connected to a portal vein infusion tube and flushed with 0.5 ml sterile saline containing heparin at 10 units ml^{-1}. An ascending vena cava catheter was inserted proximal to the renal veins, secured and connected to the vena cava infusion tube. The abdominal aorta was isolated and catheterized by advancing a PE 60 catheter 0.5 cm over a previously inserted 22-gauge needle into the descending aorta. After securing with cyanoacrylate adhesive, the opposite end of the PE 60 catheter was inserted into its infusion set tube and flushed with heparinized sterile saline.

The PE 60 jejunal dosing catheter was inserted through a puncture from a 24-gauge needle about 5cm distal to the ligament of Treitz. After securing in place with a single stitch of 4-0 polyester suture, it was connected to the intestinal infusion tubing and flushed with sterile saline.

The abdominal wall was closed with 4-0 Vicryl® and the skin closed using stainless steel wound clips. The infusion sets were sutured to the back with 2-0 silk as a single unit. Ampicillin (40 mg kg^{-1}) in sterile saline was injected into each of the parenteral catheters immediately following surgery. Animals were maintained in individual cages with catheters flushed every 1 to 2 days. Catheters were flushed with

0.5 ml sterile saline containing heparin (100 U ml^{-1}) and ampicillin (1 mg ml^{-1}).

In a group of 110 surgically prepared rats, the cannulas of 64 (58%) remained fully patent at least two weeks after surgery. Approximately 10% remain fully patent for 6 weeks.

Absorption Experiments

Surgically prepared rats were weighted daily and allowed to recover for at least 4 days. Fasted rats were dosed over 15 seconds via the jejunal catheter with dose solutions containing drug and absorption probe. Portal and aortic(systemic) blood samples were simultaneously withdrawn at specified times. Following sampling, cardiovascular equilibrium was maintained by replacement with blood from donor rats. Hematocrits did not significantly decline during our procedure. Hematocrits were 41.3 ± 0.6 prior to sampling and decreased to 40.2 ± 0.6 after sampling. Rats were allowed to recover for at least 4 days between studies.

Solutions for enteral dosing were prepared in distilled water at concentrations such that the dose volume ranged from 1 to 3 ml. Theophylline was dosed at 10 or 25 μmoles kg^{-1}. Verapamil was dosed at 10 μmoles kg^{-1}, A-79035 at 15 μmoles kg^{-1} and fluorescein at 1 μmole kg^{-1}. Tritiated water and ^{3}H-PEG-458 were dosed at 6.72×10^6 dpm kg^{-1} (3.03 μCi kg^{-1}) and 1.11×10^8 dpm kg^{-1} (50 μCi kg^{-1}), respectively. Parenteral dose solutions were prepared in saline at concentrations that resulted in dose volumes from 0.5 to 2 ml.

Assays

A protein precipitation procedure was used for all assays. To 0.4 ml acetonitrile was added 0.2 ml blood sample or standard while mixing rapidly for 5 seconds. After centrifugation at 1500 rpm for 2 min, specific aliquots of the supernatant were removed for each analysis. All HPLC analyses used 5 × 0.46 cm reverse phase Little Champ® columns (Regis Chemical Co.).

Tritiated water and ^{3}H-PEG-458 were assayed by adding 50 μl of acetonitrile-blood supernatant to 3 ml of scintillation cocktail (Insta-Gel® XF, Packard, Meriden, CT), mixing and counting (Beckman Model LS 3801). Conversion to dpm was achieved against quench correction curves. Dose solutions containing ^3H_2O or ^{3}H-PEG-458 were assayed to obtain total radioactivity administered to each animal.

Fluorescein was assayed by adding 0.2 ml of acetonitrile-blood supernatant to a test tube containing 2 ml of 10mM phosphate buffer (pH 9). After mixing, the fluorescence was measured on a Perkin-Elmer Model LS-5 spectrophotofluorometer at 514 nm after excitation at 490 nm.

Theophylline was assayed by adding 0.1 ml of acetonitrile-blood supernatant to an HPLC autosample vial containing 1 ml water and injecting 50 μl onto the HPLC column. HPLC mobile phase was acetonitrile:10 mM sodium phosphate (pH 3) (4:96) at a flow rate of 1 ml min^{-1}. Detection was by UV absorption at 273 nm.

Verapamil was assayed by adding 0.2 ml of acetonitrile-blood supernatant to an HPLC autosample vial containing 0.7 ml of a tetramethylammonium perchlorate (0.02M)-trifluoroacetic acid (0.1%) (TMAP-TFA) solution and injecting 50 μl. Mobile phase was acetonitrile:methanol:TMAP-TFA (37:5:58) at a flow rate of 1.4 ml min^{-1}. Detection was by UV absorption at 228 nm.

The 5-lipoxygenase inhibitor, A-79035, was assayed by transferring 0.20 ml of acetonitrile-blood supernatant to an HPLC autosample vial containing 0.6 ml of 5 mM acetohydroxamic acid in 50 mM phosphate (pH 7). and injecting 50 μl. HPLC mobile phase consisted of acetonitrile: methanol: 5mM acetohydroxamic acid in 50 mM phosphate at pH 7 (23:5:72). The flow rate was 1.3 ml min^{-1} with UV detection at 275 nm.

Freshly prepared and acetonitrile extracted blood standards were used to determine unknown analyte concentrations in blood samples. Calibration curve parameters were determined from a least-squares linear regression analysis ($r>0.99$) of peak area or relative fluorescence(fluorescein) vs concentration. The intra-day variability of all assays was less than 15%. Minimum quantifiable concentrations by the above described methods are about 0.5 μM, 0.1 μM, 0.05 μM and 0.025 μM for theophylline, verapamil A-79035 and fluorescein, respectively.

RESULTS AND DISCUSSION

Bioavailability of Absorption Probes

The bioavailability of tritiated water and theophylline was determined to test the premise that both absorption probes are quantitatively absorbed in conscious rat. A crossover study with four conscious rats was performed with tritiated water and theophylline at a dose of 3.03 μCi kg^{-1} and 10 μmoles kg^{-1}, respectively. On day 1, two rats received an ij dose in water and two rats received an iv dose in saline. On day 3, each rat received the same dose via the alternate route. Since the half-life of water in rat is 3.5 days (8), higher tritium background corrections were required after the second dose. Bioavailabilities (Table I) in conscious rat were 95.7 ± 3.8% for tritiated water and 97.2 ± 3.6% for theophylline. In the presence of 30% PEG-400 the bioavailability of theophylline (10 μmoles kg^{-1}) remained quantitative (106.0 ± 3.0%; n = 2). Also, in 18 independent conscious rats dosed intrajejunally with theophylline at 25 μmoles kg^{-1}, the AUC was 156.2 ± 33.9 μM hr (mean ± s.d.). With an AUC following intravenous theophylline dosing (25 μmoles kg^{-1}) in 6 additional conscious rats of 165.7 ± 16.8 μM hr, a bioavailability of 94.3% (79.3-111%; 95% confidence interval) is calculated. Therefore, the current model substantiates the premise that F_G is not significantly different from unity for these absorption probes in conscious rat.

Drug Absorption

Using Eq 8, an estimate of the fraction of a dose reaching the portal blood (F_G) can be achieved by simultaneously dosing drug and absorption probe and sampling at the same site and time. It is necessary to assay whole blood to account for the amount of drug that may associate with the cellular components of blood.

A-79035 - Both the theophylline and tritiated water absorption probes were simultaneously dosed with A-79035. As shown in Table II, both probes gave comparable values of F_G for A-79035. This agrees with bioavailabilities consistently greater than 80% and indicate little or no hepatic first-

Table I. Bioavailability of Theophylline and Tritiated Water in Conscious Rat

Rat ID	Weight (g)	AUC (0-4 hr) Theophylline (μM hr) ij	Theophylline (μM hr) iv	^3H_2O (dpm hr l^{-1}; $\times 10^{-6}$) ij	^3H_2O (dpm hr l^{-1}; $\times 10^{-6}$) iv	Bioavailability, % Theophylline	Bioavailability, % ^3H_2O
Aqueous Dose							
22	489	68.8	73.1	56.9	66.1	94.1	86.1
23	406	74.9	70.9	74.4	72.6	105.6	102.4
24	458	67.0	75.8	65.8	69.5	88.4	94.6
27	385	71.6	71.1	61.4	61.7	100.7	99.7
Mean ± s.e.						97.2 ± 3.8	95.7 ± 3.6
30% PEG-400 Dose							
45	325	70.3	68.2			103.1	
53	288	76.8	70.3			109.0	
Mean ± s.e.						106.0 ± 3.0	

pass elimination for A-79035. Typical portal-systemic concentration-time profiles for A-79035 are shown in Figure 1. In a separate study, the presence of 30% PEG-400 in the A-79035 dose solution did not affect the extent of A-79035 absorption (F_G = 0.88 ± 0.06 with a theophylline absorption probe) but did affect the rate of absorption as discussed below.

Verapamil - The bioavailability of verapamil in various animal species is typically 10-20% (9). The low bioavailability is associated with a high hepatic first-pass effect (0.8-0.9). If this is the case, verapamil absorption would be expected to be nearly complete. We estimated the F_G of verapamil at 10 μmole kg^{-1} in conscious rats by coadministering it with theophylline as an absorption probe and applying Eq 8. The portal-systemic concentrations of two rats are shown in Figure 2. Even though the animals differed significantly in their portal-systemic gradients, the absorption probe, in this case theophylline, corrects for these differences. The verapamil ΔAUC values were 0.654 and 2.19 μM hr, respectively for rats 1 and 2. The corresponding theophylline ΔAUC's were 0.704 and 2.12. Since both compounds were dosed identically (10 μmoles kg^{-1}) the calculated F_G (Eq 8) for verapamil was 98.0 ± 5.2% indicating complete intestinal absorption. Verapamil absorption was rapid and complete within 1 hour as evidenced by the merging of the portal and systemic concentration-time profiles in Figure 2. Relative to an intravenous dose of verapamil in these rats, the bioavailability was 21.4 ± 3.2%. These data agree with previous findings that verapamil is well absorbed but susceptible to a hepatic first-pass effect of about 0.8 in rat (10). A principal enzyme in the metabolism of verapamil in humans is cytochrome P450-3A (11). Although rat intestine contains cytochrome P450-3A, our result suggests that this enzyme does not significantly metabolize absorbed verapamil following acute dosing.

Absorption Rate Constants

Assuming transport across the intestinal epithelium follows first-order kinetics, absorption rate constants (k_a) can be estimated from the slope of a linear regression analysis of F_G^t vs time using initial time points ($F_G^t < 0.2$ dose) as shown in Figure 3. It is also assumed that the compound is stable to luminal and intestinal degradation or metabolism. If intestinal degradation or metabolism is suspected, the effective dose (F_G^∞) is the total empirical fraction of the dose that reaches the portal blood. The absorption rate constant is linearly related to permeability and is useful for correlating intestinal absorption with formulation factors (osmotic gradient), physicochemical properties of a compound (partition

Table II. Absorption of A-79035 in Conscious Rat. Comparison of Tritiated Water and Theophylline Absorption Probes

Rat ID	Weight (g)	ΔAUC A-79035[a] (μM hr)	ΔAUC Theophylline[a] (μM hr)	ΔAUC ^3H_2O[a] (dpm hr l^{-1}; $\times 10^{-6}$)	F_G (A-79035) Theophylline	F_G (A-79035) ^3H_2O
11	286	3.38	2.78	1.76	0.86	0.81
15	244	3.78	2.80	2.17	0.78	0.90
51	322	3.14	2.49	1.49	0.94	0.84
66	350	2.36	1.99	1.27	0.84	0.79
15[b]	282	3.58	2.98	1.81	0.89	0.80
Mean ± s.e.					0.86 ± 0.03	0.84 ± 0.02

[a] Dosages were 15 μmoles kg^{-1}, 10 μmoles kg^{-1} and 6.73 × 10^6 dpm kg^{-1} for A-79035, theophylline, and tritiated water, respectively.
[b] Repeat study with rat 156 days after first study (not used in calculation of mean).

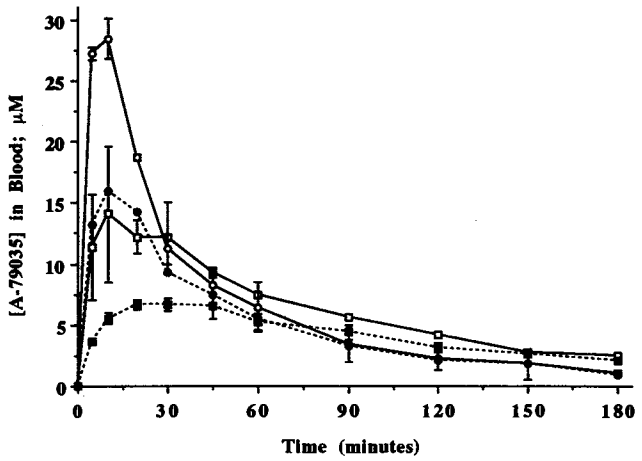


Fig. 1. Portal (open symbols) and systemic (closed symbols) A-79035 blood concentrations following an intrajejunal dose of 15 μmoles kg^{-1} in water (circles) or 30% PEG 400 (squares). Using theophylline as the absorption probe, F_G = 0.86 ± 0.03 (n=4) in water and 0.88 ± 0.06 (n=4) with 30% PEG 400.

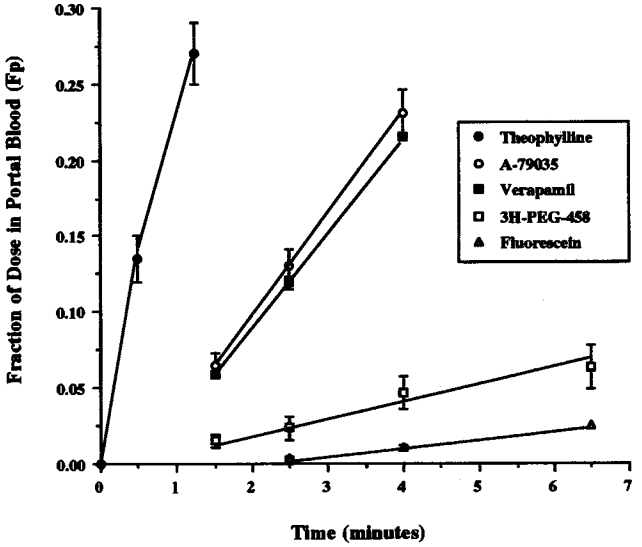


Fig. 3. Flux of various solutes across the intestinal epithelium of conscious rats. Each point represents the mean and standard error of at least 3 determinations.

coefficient, solubility) and physiological factors (anesthesia, mesenteric blood flow, drug dependent water flow). For example, PEG-400, which is a commonly used pharmaceutical vehicle to aid drug solubility, is known to reduce the absorption rate of carbamazepine in rabbits (12). As shown in Table III, when theophylline and A-79035 were dosed in the presence of 30% PEG-400, their respective absorption rate constants were significantly reduced ($p>0.05$) to 21% and 37% that of aqueous dose solutions. Reduction of absorption rates in the presence of 30% PEG-400 is presumably due to two factors; reduced membrane partitioning (log D) and reverse solvent drag (water efflux from intestine to lumen) due to the high luminal osmolarity of 30% PEG-400 (2200 mOs).

The theophylline and A-79035 absorption rates in anesthetized rats were 60% and 52%, respectively, those in conscious rats. Presumably, reduced mesenteric blood flow caused by anesthesia (13,14) slows the absorption of rapidly absorbed drugs (15). Additional absorption rate constants are shown in Table III and can be ranked in the order theophylline, water > A-79035, verapamil > PEG-458 > fluorescein.

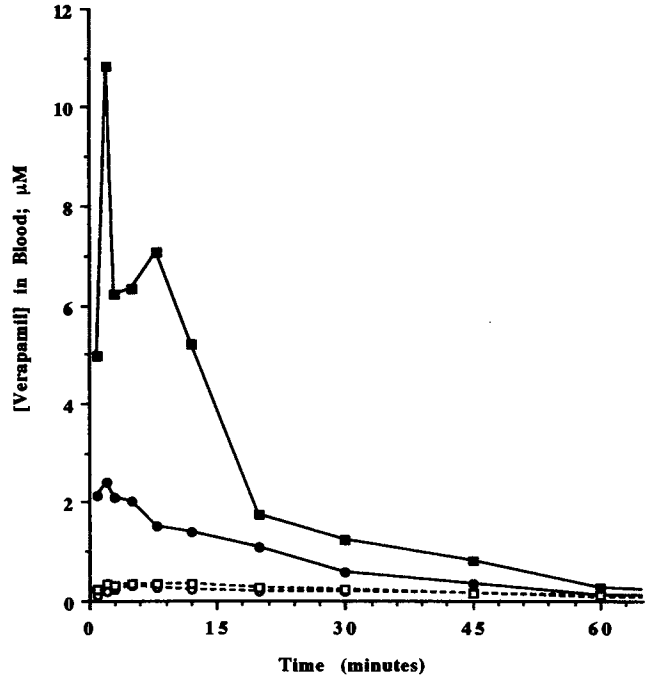


Fig. 2. Portal (closed symbols) and systemic (open symbols) blood verapamil concentrations following an ij dose of 10 μmoles kg^{-1}. Circles are Rat 1 and squares are Rat 2.

DISCUSSION

While it is recognized that the current model requires microsurgical techniques, the daily maintenance of cannula patency and an additional assay for the absorption probe, there are several advantages of this method in estimating drug absorption in conscious rats. The model allows quantitative evaluation of the intestinal absorption barrier in small, inexpensive and pure bred animals by directly mea-

Table III. Absorption Rate Constants in Conscious Rat

	k_a, (min^{-1})	Log D
Theophylline		
Water Vehicle	0.209 ± 0.014 (5)[a]	−0.05
30% PEG-400	0.051 ± 0.013 (5)	−0.16
Anesthetized[b]	0.133 ± 0.029 (5)	
A-79035		
Water Vehicle	0.059 ± 0.005 (4)	1.71
30% PEG-400	0.024 ± 0.005 (4)	0.90
Anesthetized[b]	0.035 ± 0.008 (4)	
Water	0.211 ± 0.064 (16)	
Verapamil	0.057 ± 0.003 (3)	
^{3}H-PEG 458	0.010 ± 0.002 (4)	
Fluorescein	0.005 ± 0.001 (2)	

[a] Mean ± s.e. Number of animals parentheses.
[b] Nembutal/ketamine anesthesia with water dose vehicle.

suring the amount of compound that reaches the portal blood. Hepatic first-pass effects can also be estimated provided that bioavailability is known. If chronicity or anesthesia are issues of concern, repeat studies on the same animal are routine. Conscious animal studies also require less time than anesthetized studies since anesthesia, surgery and post-surgical stabilization are eliminated. Of equal or greater significance, measurement of portal blood flow rate is not necessary. The use of measured (electronic flow probe) values for portal blood flow may, in fact, result in underestimates of fractional absorption since estimates of drug concentrations in portal blood may be biased, a shortcoming eliminated through the use of absorption reference compounds.

The fraction of a dose which reaches the portal blood (F_G) provides phenomenological information regarding absorption. High F_G values simply reflect a well absorbed compound. Low F_G may signify several possibilities: (a) poor absorption, (b) gut metabolism or degradation, or (c) intestinal lymphatic transport. The fact that gut metabolism reduces F_G makes this model a useful tool in identifying pre-portal biotransformations. If putative metabolites are known and can be assayed, then the observation of portal-systemic metabolite gradients would confirm gut metabolism. Diversion of significant amounts of intestinally absorbed drug to lymph is unlikely since such effects generally require octanol/water partition coefficients of 10^6 or greater (16). Lipophilicity of this magnitude is several orders of magnitude greater than that of the majority of drugs; intentional efforts to divert absorbed drug to the lymphatic system have met with very limited success (17).

Absorption rate constants derived directly from the net flux of drug into the portal vein are very useful in evaluating formulation (enhancers, controlled release, nutrients, etc.) and physiological (anesthesia) effects on drug absorption.

In conclusion, a conscious rat absorption model has been developed which estimates the rate and extent of intestinal absorption to portal blood without need for simultaneous measurement of portal blood flow rates. Such results may prove helpful in specifically identifying potential barriers to the oral delivery of therapeutic agents.

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