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Research Paper

Dog Colonoscopy Model for Predicting Human Colon Absorption

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Purpose. This study was conducted to develop and validate a dog colon model that predicts colon permeability in humans.
Methods. The following compounds were studied: Class 1 highly soluble (HS)/highly permeable (HP): aminophylline, propranolol, CP-409092; Class 2 LS/HP: nifedipine; trovafloxacin, sertraline; Class 3 HS/LP: azithromycin, atenolol, CP-331684, CP-424391; Class 4 LS/LP: CJ-13610. Administration to dogs was made 30 cm cranial to the anal sphincter with a lubricated Schott Model VFS-5 flexible endoscope. The bioavailability of the compound following the colon administration in dogs, relative to the same formulation administered orally (relative bioavailability), was determined.
Results. Except for atenolol, a small hydrophillic molecule, the relative bioavailability from administration to the colon of the dog correlated well with the following compound properties: high solubility and high, passive permeability > high solubility, low permeability > low solubility, high, passive permeability ~ low solubility, low permeability.
Conclusion. The dog colon model is proposed as a surrogate for human intubation studies when the controlled release candidate falls in BCS Classes 2 (LS/HP), 3 (HS/LP), and 4 (LS/LP). However, no human intubation or dog colon studies are required for Class 1 (HS/HP), as these compounds are likely to be well absorbed from the colon.

KEY WORDS: aminophylline; atenolol; azithromycin; biopharmaceutics classification scheme; CJ-13610; CP-331684; CP-409092; CP-424391; nifedipine; propranolol; sertraline; trovafloxacin.

INTRODUCTION

Controlled release (CR) formulations are rapidly becoming a standard part of a development candidate's portfolio. A well-formulated CR formulation can overcome a compound's pharmacokinetic and/or pharmacodynamic inutility by extending exposure and decreasing adverse side effects. For long-duration CR formulations, a significant portion of the payload is released while the formulation resides in the colon or large bowel. If solubility and/or permeability in the colon are not optimal, then success will be defined by the dose and the residence time in that organ. That is, the dosage form and/or the delivered drug particles must reside in the large intestine long enough for their complete dissolution and absorption.

However, one simply cannot assume that a compound formulated in an immediate release (IR) dosage form, administered orally, and resulting in good absorption will be well absorbed when formulated in a long-duration CR dosage form. When compared with the small intestine, the colon has a shorter length, smaller surface area, more bacterial activity, less active transport, more restrictive tight junctions (less paracellular transport), less water, less bile acid and lecithin, higher alkalinity, higher viscosity, and less mixing (1,2). Therefore, it is not surprising to find it difficult to predict human colonic absorption from the usual preclinical and clinical studies.

Bioavailability of the compound administered to the large bowel, relative to the bioavailability after oral administration, is defined as RBA. Complete bioavailability—for bioequivalence strategies, RBA~80%—cannot be assumed. A quick survey of the literature revealed that out of 16 compounds examined for their potential as CR candidates, half had an RBA of less than 30% (2,3). Some measurement of the CR candidate's permeability in the human colon seems prudent. However, due to the increased number of candidates for CR development, a resource- and time-sparing predictive model for human colon permeability would be useful.

Several *in vitro* and *in vivo* models have been explored for this purpose. Flux across Caco-2 cell monolayers was shown to correctly rank the absorption of several highly water-soluble compounds in the human colon (4). But for the preclinical evaluation of clinical dosage forms, *in vivo* data are mandatory and the dog and minipig are the most commonly studied species. Despite any perceived advantage of the minipig, the dog remains the species of choice for extended release for development (5–7).

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Colonic absorption of full-sized ("clinical") CR dosage forms have been studied in the dog instrumented with a Thiry-Vella fistula (8). Solutions of CR candidates were similarly studied in dogs instrumented with a modified "vascular access port" (6,9) and by oral administration of a remotely triggered device that would release its payload when in the region of interest (10). The first two methods, although direct and useful, require surgery and regular maintenance. The last is noninvasive, but has the following drawbacks: (1) it is expensive; (2) it requires specialized equipment and licensing; (3) specific information about colonic absorption is difficult to glean from such a study without some independent confirmation of location in the GIT (such as scintigraphy).

Sutton and coworkers (11) reported colonoscopy as a simple, resource- and time-sparing alternative to these methods. In that preliminary report, aminophylline and enalapril HCl solutions were administered into the colon of Beagle dogs. This report extends that early work, validating the model by comparing numerous additional dog colon studies with clinical data.

METHODS

Materials

Compounds spanning the Biopharmaceutics Classification Scheme (BCS) (12) were selected. Three compounds (aminophylline, propranolol, CP-409092) were classified as Class 1, i.e., "highly soluble/highly permeable" (HS/HP); three compounds were Class 2 (LS/HP): trovafloxacin, sertraline and nifedipine; four compounds were Class 3 (HS/LP): atenolol, azithromycin, CP-331684, CP-424391; and one compound was Class 4 (LS/LP): CJ-013610. Aminophylline (the "salt" or complex of theophylline and ethylenediamine), atenolol HCl, and propranolol HCl were purchased from Sigma (St. Louis, MO). Azithromycin, micronized nifedipine, sertraline, trovafloxacin mesylate, CP-331684[1] (a β3 agonist for the treatment of obesity), CP-409092[2] (a $GABA_a$ partial agonist developed for the treatment of anxiety), CP-424391 tartrate[3] (a growth hormone secretagogue in clinical trials for the treatment of frailty (13,14)), CJ-013670 mesylate[4] (a 5-lipoxygenase inhibitor with oral activity in animal models of inflammation and airway obstruction (15)) were obtained from Pfizer, Inc. Selected biopharmaceutical properties of the 11 compounds studied are listed in Table I.

Solubility Determination

According to the BCS Guidelines, a compound is considered "highly soluble" when the largest dose is soluble in a volume of 250 mL water through the pH range of 1–7.5 (12). As shown in Table I, the solubility of aminophylline is 200 mg/mL, and both propranolol HCl and atenolol HCl are "soluble" in water (16). The solubility of nifedipine is about 0.011 mg/mL. The remaining compounds examined in this report are weak bases/zwitterions, and their pH solubility profiles were determined by using classical methods (17). Based on these solubilities and a "best-guess" of the clinical dose, a solubility classification was made (Table I). A table of high-performance liquid chromatography (HPLC) conditions for the solubility determinations is shown in the Appendix.

Permeability Determination

Depending on the availability of data, the permeability classification of a compound according to BCS may be supported by clinical studies [e.g., absolute bioavailability studies (i.e., IV and oral), or mass balance studies]. When clinical data are not available, a preliminary estimate of a compound permeability classification can be determined from such *in vitro* models as cell monolayer and rat intestinal models. In our laboratory, the rat single pass intestinal perfusion (SPIP) was used to preliminarily classify compounds (18,19) (Table I).

Formulations

Except for nifedipine, compounds were administered orally and to the colon as aqueous solutions, or (oral only) as rapidly dissolving immediate release formulations. In some cases, the weak base was made soluble by adjusting the pH. For example, the CP-409092 solution was prepared by adding the compound to a beaker and bringing to a 30.0 mL volume of 0.5% methylcellulose, followed by a pH adjustment to 6.9 with 0.25 M NaOH. The drug remained in solution during the transition in pH. The 1 mg/mL nifedipine suspension was prepared in a 0.5% methylcellulose. The solution formulation consisted of 2 mg/mL nifedipine in (1:2:3:4) Tween 80/cremaphor EL/propylene glycol/water (20).

Animal Studies

The *in vivo* studies described here were reviewed and approved by the Pfizer Groton Institutional Animal Care and Use Committee, and the research adhered to the Principles of Laboratory Animal Care (NIH publication #85-23, revised in 1985). Studies were completed in purpose-bred, Class A adult (1- to 6-year-old) male and female Beagle dogs (Marshall Farms, North Rose, NY, USA; weight range 9–13 kg). Orally administered capsule and tablet formulations were followed by a 50-mL tap water gavage. Liquid formulations were orally administered with a syringe, through the same length and type of tubing used in the colonoscopy study, inserted within a stomach gavage tube. Tubing was flushed to ensure complete delivery. Separate validation studies were conducted to ascertain >95% of the intended dose was delivered using these methods (data not shown). Blood was collected from the jugular vein before dosing and at specified intervals afterwards (e.g., 0.5, 1, 2, 4, 6, 8, and 24 h). Blood was then centrifuged, and plasma or sera was separated.

[1] (4-(2-(2-(6-Aminopyridin-3-yl)-2(*R*)-hydroxyethylamino)-ethoxy)-phenyl)-acetic acid.

[2] CP-409092: 4-Oxo-4,5,6,7-tetrahydro-1*H*-indole-3-carboxylic acid (4-methylaminomethyl-phenyl)-amide.

[3] 2-Amino-*N*-[2-(3′*R*-benzyl-2-methyl-3-oxo-2,3,3a,4,6,7-hexahydro-pyrazolo[4,3-*c*]pyridin-5-yl)-1*R*-benzyloxymethyl-2-oxo-ethyl]-isobutyramide L-tartrate.

[4] 4-[3-{4-(2-Methylimidazol-1-yl)phenylthio}]phenyl-3,4,5,6-tetrahydro-2*H*-pyran-4-carboxamide methanesulfonate.

Table I. Biopharmaceutical Properties of the Compounds Studied in this Report

Compound	BCS	MW (Da)	Solubility[a] (mg/mL)	log P[b]	pK_a	Rat k_a (min^{-1})
Aminophylline	HS/HP	420	200	−0.1[c]	NA	0.034 ± 0.003
Atenolol	HS/LP	266	>1	−2.1	9.6	0.006 ± 0.003
Azithromycin	HS/LP	749	4.5	~2	~9	<0.003
Nifedipine	LS/HP	346	0.011	2.07	NA	HP[d]
Propranolol	HS/HP	259	>1	2.5	9.4	0.035 ± 0.005
Sertraline	LS/HP	343	≈0.37	4.88	9.1	0.07 ± 0.02[e]
Trovafloxacin	LS/HP	513	0.050	1.9	5.6, 9.5	ND
CJ-013610	LS/LP	490	<0.1	2.9	7.3	0.003
CP-331684	HS/LP	331	3.3	−2.2	3.9, 5.5, 8.1	0.006 ± 0.017
CP-409092	HS/HP	334	25	1.8	9.1	0.014 ± 0.003
CP-424391	HS/HP	656	11	2.0	7.7	0.011 ± 0.018

[a] Aqueous thermodynamic, at pH 6.5, RT.
[b] Octanol/pH 7 water.
[c] Calculated by using Moriguchi *et al.*'s method (43).
[d] Hoyo-Vadillo *et al.* (44).
[e] Permeability of sertraline is complicated by its apparent active transport. Sertraline HCI was shown to be a substrate for active transport in Caco-2 (J. Bennett, personal communication).
MW: Molecular weight; NA: not applicable; ND: not determined.

The plasma/sera was immediately frozen at −20°C until the compound of interest was assayed (usually within a week). Access to food was permitted after the 8-h sample collection. Animals had free access to water during the experiment.

The colonoscopy model was used as previously described (11,21). Beagle dogs were prepared for the colonoscopy procedure with at least two "training" (sham) studies, during which they learned to accept the endoscope. Early experience revealed that residual material in the DC interfered with the unobstructed placement of the colonoscope. Therefore, for 3 days before the study day, the normal chow diet was removed from dogs and they were provided a liquid diet (#5033RL-375/2, Clinicare© Canine Liquid Diet, North Chicago, IL, USA). All the dogs were fasted for 24 h immediately before the study. Animals were manually restrained on their left side and calmed by frequent petting and soothing speech. A lubricated Schott Model VFS-5 flexible endoscope with an external diameter of 9.9 mm was carefully inserted through the anal sphincter and advanced a distance of 30 cm. If significant resistance was met, the scope was withdrawn, typically followed by almost immediate defecation. The scope could then be easily advanced without resistance. The dose was administered as a 5-mL bolus via a syringe attached to PE-200 tubing filled with the formulation, inserted via the biopsy channel. After complete delivery of the compound, the tubing was flushed to ensure complete delivery, and the endoscope was slowly retracted. Separate validation studies were conducted to ascertain >95% of the intended dose was delivered by using these methods (data not shown). Because feeding sometimes can trigger a generalized propulsive movement along the entire GIT, food was withheld until after the absorption phase could be reasonably expected to be over (by the 8-h sampling point). The rest of each study was completed as described for the oral studies.

Assays

For all compounds except CP-409092, individual samples were assayed for kinetic analysis; however, samples with CP-409092 were collected until preliminary studies suggested concentrations would be <LLOQ, and then "pooled" according to the method of Hop *et al.* (22) and assayed. HPLC analysis of trovafloxacin (23) aminophylline (24), nifedipine (25), azithromycin (26), CP-331684 (19), CP-409092 (27), CP-424391 (28), and CJ-013670 (15) in dog plasma or serum was completed by using published assays. Details are presented in Table II.

Pharmacokinetics

Area under the concentration time profile from "time 0" to the last detectable concentration ($AUC_{0-\tau}$) was calculated by using the mix log-linear trapezoid method. Extrapolated AUC was calculated by C_{last}/L_z, where C_{last} is the last detectable concentration and L_z is the terminal elimination rate constant. $AUC_{0-\infty}$ is the sum of the two areas. Relative bioavailability (RBA) was calculated by the ratio of the dose-corrected AUC of the colon [or ileal–cecal junction (ICJ)] studies and the oral (gavaged or duodenal) studies. The k_a values were either calculated from simultaneous fitting of IV (data not shown) and extravascular data, or from the initial linear portion of the log absorption *vs.* time plots, which in turn were calculated from either deconvolution or Wagner–Nelson methods (Kinetica version 4.01-4.3; Innaphase, Philadelphia, PA, USA). The concentrations of the CP-409092 "pooled" samples were determined, and the colon sample values were expressed as a percentage of the oral sample values. According to Hop *et al.* (22), this would approximate the bioavailability for the colon administered dose relative to the orally administered dose (RBA).

Statistics

Arithmetic mean (AM) and standard deviation (SD) were calculated for T_{max}, and geometric mean (GM) and percent coefficient of variation (CV) were calculated for C_{max} and $AUC_{0-\infty}$. Differences in pharmacokinetic parameters obtained from the two routes of administration were

Table II. Assay Conditions for Plasma/sera Matrices Containing the Compounds Studied

Compound	Internal standard	Column[a]	Flow (mL/min)	Detection (nm)	Injection (μL)	Compound Tr (min)	Mobile Phase
Aminophylline	Theobromine	150 mm Waters C18 Novapak	1	280	25	3.3	A[b]
Atenolol	Metoprolol	300 mm Waters C18 Bondapak	1	240/300[c]	10	2.3	C[d]
Azithromycin	CP-067094[e]	150 mm 3 M Z-RP	1	EC	15	9.6	D[f]
Nifedipine	11-Ketoprogesterone	C18, 5 μm	1.5	238	200	7.0	E[g]
Propranolol	Phenytoin	150 mm Zorbax C18	1.5	215	25		B[h]
Trovafloxacin	CP-102372[i]	150 mm Waters C18 Novapak	0.7	275	100	5.8	J[j]
CP-409092	CP-409095[k]	Zorbax Eclipse XDB-C8, 4.6 × 150 mm, 5 μm	1	246	20	11	H[l]
CJ-13610	CJ-13454[m]	150 mm Waters C18 Symmetry®	0.5	MS[n]	20	2.6	L[o]
CP-331684	CP-345529[p]	C18 column (3 μm; 4.6 × 33 μm)	2	290/370[c]	25	4	G[q]
CP-424391	CP-395477	Keystone Inertial ODS-2 2.1 × 50 mm 5 μm C18	NA	MS[r]	40	5.7	I[s]

[a] All were run at ambient temperature.
[b] A: 5:95 acetonitrile/0.1 M sodium acetate, pH 4.0, using glacial acetic acid.
[c] Fluorescence detector: excitation wavelength/emission wavelength.
[d] C: Acetonitrile/methanol/0.02 M sodium phosphate buffer (0.1% SDS): 35:15:50 (v/v/v) (45). C: 50:50 0.01 M KH_2PO_4/methanol; pH 4.0.
[e] CP-067094: 11-(4-dimethylamino-3-hydroxy-6-methyl-tetrahydro-pyran-2-yloxy)-2-ethyl-3,4,10-trihydroxy-13-(5-hydroxy-4-methoxy-4,6-dimethyl-tetrahydro-pyran-2-yloxy)- 3,5,8,10,12,14-hexamethyl-6-prop-2-ynyl-1-oxa-6-aza-cyclopentadecan-15-one.
[f] D: Acetonitrile/sodium phosphate buffer (26). 75:25 0.05 M KH_2PO_4/acetonitrile with 0.1% TFA; pH 4.0.
[g] E: Acetonitrile/methanol/tetrahydrofuran/ "buffer": 200:200:15:585. E: 90:10 water with 0.1% TFA/acetonitrile.
[h] B: Gradient: initial—75:25 water with 0.1% TFA/acetonitrile, 0–6 min; 6–9 min ramp to 40:60 water with 0.1% TFA/acetonitrile.
[i] CP-102372-01: 7-(6-amino-2-methyl-3-aza-bicyclo[3.1.0]hex-3-yl)-1-(2,4-difluoro-phenyl)-6-fluoro-4-oxo-1,4-dihydro-[1,8]naphthyridine-3-carboxylic acid.
[j] J: The mobile phase was prepared by mixing 5.3 mL of 40% (w/w) tetrabutyl ammonium hydroxide and 1 vial of Waters PIC reagent D-4 (dibutylamine phosphate in water) to 830 mL of 0.04 M H_3PO_4. The volume was brought to 1 L with acetonitrile. The resulting pH 2.4 solution was adjusted to pH 3.0 with 5.0 N NaOH.
[k] CP-102372: 7-(6-amino-2-methyl-3-aza-bicyclo[3.1.0]hex-3-yl)-1-(2,4-difluoro-phenyl)-6-fluoro-4-oxo-1,4-dihydro-[1,8]naphthyridine-3-carboxylic acid.
[l] H: buffering reagent (1 M sodium carbonate, pH 11); mobile phase "H1" [1% isopropanol in 2 mM ammonium acetate (pH 3.5) with 0.1% TEA], mobile phase "H2" (1% isopropanol in acetonitrile).
[m] CJ-013454: 4-{3-fluoro-5-[4-(2-methyl-imidazol-1-yl)-benzyloxy]-phenyl}-tetrahydro-pyran-4-carboxylic acid amide.
[n] Positive ions were monitored for the quantification of CJ-13,610 (m/z 394.1) and CJ-13,454 (m/z 410.3).
[o] L: Mobile phase solvents were: solvent L1 = 5 mM ammonium acetate, with 1% isopropyl alcohol per liter of mobile phase; solvent L2 = acetonitrile, with 1% isopropyl alcohol per liter of mobile phase. The gradient was 0–3.0 min, 100% L1 to 0% L1, at 3.1 min switch back to 100% L1.
[p] CP-345529: 3-(4-{2-[2-(6-amino-pyridin-3-yl)-2-hydroxy-ethylamino]-ethoxy}-phenyl)-propionic acid.
[q] G: 16% acetonitrile, 84% of 50 mM monobasic sodium phosphate with 5 mM octane sulfonic acid, pH 3, with phosphoric acid.
[r] Mass spec ions monitored at m/z 526.
[s] I: Compounds of interest were eluted from the column using a gradient elution program (Table S1, Appendix, Electronic Supplementary material is available for this article at 10.1007/s11095-006-0252-3 and is accessible for authorized users). The mobile phases consisted of I1 = 10:90:0.01 (v/v/v) acetonitrile/water/formic acid and I2 = 90:10:0.01 (v/v/v) acetonitrile/water/formic acid.

considered significant when two-tailed Student's t test results were $p < 0.05$.

RESULTS

For easy future reference, results for compounds are listed in Table III in alphanumeric order.

Class 1: HS/HP

Aminophylline (HS/HP)

Colonic absorption of 10 mg/kg aminophylline was incomplete in dogs that defecated during the first 0.5 h of the study. As shown in Table III, the apparently truncated residence time had a profound effect on C_{max} and $AUC_{0-\infty}$. For example, $AUC_{0-\infty}$ in dogs with defecation was about half of the $AUC_{0-\infty}$ in dogs without defecation. For all compounds in this report, only studies where no defecation occurred during the compounds' absorptive phase were therefore considered for further data analysis. The pharmacokinetics of theophylline in the dog, following the colonic and oral administration of 10 mg/kg aminophylline, is summarized in Table III. The aminophylline k_a after colon administration was about 12% of the k_a after oral gavage. The AUC of theophylline after colonic administration of aminophylline (104 μg h/mL) was 87% of the AUC after oral dose in the same dogs (128 μg h/mL). One could conclude from the T_{max} and k_a data that aminophylline was slowly, but completely absorbed from the colon.

Table III. Results of Oral and Colon Administration of the Model Compounds in Dogs

Compound	Formulation	Route	Dose (mg/kg)	*n*	T_{max} (h)	C_{max} (μg/mL)	$AUC_{0-\infty}$ (h μg/mL)	RBA[a] (%)	k_a[b] (h^{-1})
Aminophylline	Solution	Oral	10	3	0.8 (0.3)	13.8 (10.1)	128 (10.4)	–	2.99
Aminophylline	Solution	Colon	10	3	2 (1)	7.5 (38)	104 (42)	87 (0.4)	0.35
Aminophylline	Solution	Colon	10	4	1.4 (1.2)	3.9 (86)	64 (50)[c]	50	
Atenolol	Solution	Oral	10	3	0.8 (0.3)	5.18 (8.8)	21.8 (4.1)		0.62
Atenolol	Solution	Colon	10	3	3.7 (3.8)	1.2 (28)	13.5 (17)	60 (9.8)	0.022
Azithromycin	Solution	Oral	2.5	6	0.9 (0.6)	0.177 (49)	2.18 (34)	–	1.5 (1.4)
Azithromycin	Solution	Colon	25	3	4.3 (6.6)	0.16 (37)	2.33 (96)	8.7	1.4
Enalapril[d]	Solution	Oral	1	4	5.0 (2.6)	0.225 (29)	2.37 (5.4)	–	0.60
Enalapril	Solution	Colon	1	4	6.7 (1.2)	0.039 (56)	0.735 (52)*	31	0.17
Nifedipine	Suspension	Oral	1[h]	4	0.6 (0.1)	0.033 (59)	0.103 (59)	–	ND
Nifedipine	Suspension	Colon	2	4	1.0 (0.00)	0.008 (33)	0.036 (76)	35	ND
Nifedipine	Solution	Oral	2	4	0.9 (0.8)	0.12 (66)	0.170 (62)	–	2.2
Nifedipine	Solution	Colon	2	4	0.9 (0.1)	0.024 (57)	0.158 (48)	93	0.35
Propranolol	Solution	Oral	4	3	1.5 (0.5)	1.12 (24)	4.58 (4.3)	–	0.47
Propranolol	Solution	Colon	4	3	1.7 (0.3)	0.61 (10)	4.51 (15)	100 (18)	1.03
Sertraline	Solution	Oral	5	3[e]	2.8 (0.5)	0.079 (18)	0.374 (5.9)	–	ND
Sertraline	Supersaturated[g]	Colon	2	3[f]	3.3 (1.5)	0.028 (47)	0.035 (47)*	9.3 (4.4)	ND
Trovafloxacin	Solution	Oral	5	4	0.7 (0.4)	0.78 (65)	3.69 (61)	–	ND
Trovafloxacin	Supersaturated[g]	Colon	5	4	2.7 (2.1)	0.18 (50)	1.03 (56)	25 (1.5)	ND
CJ-13610	Solution	Oral	4.5	4	1.4 (0.7)	1.13 (63)	5.89 (38)	–	4.3
CJ-13610	Supersaturated[g]	Colon	5	4	1.8 (0.5)	0.38 (42)	2.79 (43)	47	0.95
CP-331684	Solution	Oral	5	2	2, 2	1.7, 2.3	9.3, 14.6	–	0.22
CP-331684	Solution	Colon	5	4	0.8 (0.3)	1.04 (48)	3.21 (19)*	27	NC
CP-409092	Solution	Oral	10	6	NC	NC	87.0 (37.3)		NC
CP-409092	Solution	Colon	10	6	NC	NC	54.8 (45.9)	69 (33)	NC
CP-424391	Solution	Oral	1	5	0.6 (0.3)	0.090 (51)	0.239 (40)	–	20
CP-424391	Solution	Colon	1	5	1.6 (1.3)	0.050 (48)	0.223 (31)	93	19

For T_{max}, arithmetic mean (standard deviation), for C_{max} and $AUC_{0-\infty}$, geometric mean (coefficient of variation) are shown.
*Significantly different from oral route, $p < 0.05$.
[a] Bioavailability of the compound via the colon route, relative to that of the oral route (dose-adjusted).
[b] k_a: Absorption rate constant, calculated by deconvolution or Wagner-Nelson methods.
[c] Early defecation resulted in incomplete absorption.
[d] Sutton *et al.* (11).
[e] One dog had emesis before the 0.5 sample collection, was excluded from means analysis.
[f] One dog defecated 10 min after dosing was excluded from means analysis.
[g] "Supersaturated" reflects the almost certain likelihood that the solution precipitated after administration (see text for details).
[h] Pharmacokinetic results normalized to a 2 mg/kg dose.
ND: Not determined since the formulation either was a suspension, or precipitation from a solution was likely. NC: not calculated (see text for details)

Propranolol (HS/HP)

The average pharmacokinetic results for propranolol after oral and colon administration of 4 mg/kg propranolol HCl to three fasted Beagle dogs, in a crossover fashion, are summarized in Table III. The average plasma propranolol profiles in dogs were similar following the administration by either route. The AUC of propranolol HCL administered to the colon (4.51 μg h/mL) was ~100% of the AUC following the same dose administered orally (4.58 μg h/mL). The k_a for propranolol after administration to the colon (1.03 h^{-1}) was faster than after oral administration (0.47 h^{-1}). This was in agreement with previous reports in the dog (29) and human (30), and may be attributed to regional differences in intestinal metabolism.

CP-409092 (HS/HP)

A 10 mg/kg dose of CP-409092 was administered to six dogs orally and to the colon in a crossover study. Because the samples collected from dogs following the administration of CP-409092 were pooled, concentration–time profiles were not possible. As shown in Table III, the pooled concentrations of CP-409092 administered via the colon route (54.8 μg/mL) was 69% of the pooled concentration following oral administration (87.0 μg/mL). This 69% approximates the bioavailability for the colon administered dose relative to the orally administered dose (RBA) (22).

Class 2: LS/HP

Sertraline (LS/HP)

A 5 mg/kg dose of sertraline HCl was administered orally to four dogs in a crossover fashion, whereas a 2 mg/kg dose was administered to the colon. The results following sertraline HCl administration are summarized in Table III. Compared to oral administration, sertraline absorption from the colon was slower (T_{max}: 3.3 *vs.* 2.8 h, C_{max}: 28 *vs.* 79 ng/

mL) and less complete ($AUC_{0-\infty}$: 35 *vs.* 374 ng h/mL). This represented RBA of 9.3%.

Nifedipine (LS/HP)

Nifedipine was administered orally to four dogs as a 1 mg/kg suspension dose and a 2 mg/kg solution dose. It was also administered to the colon in 2 mg/kg doses as the suspension and solution. Results of nifedipine administration were normalized to the 2 mg/kg dose and are summarized in Table III and in Fig. 1. The nifedipine AUC was ranked in the following order: oral solution (0.170 μg h/mL) > colon solution (0.158 μg h/mL) > oral suspension 0.103 μg h/mL) > colon suspension (0.036 μg h/mL). The RBA of colon-administered nifedipine solution formulation was 93% (relative to the orally administered solution formulation). Therefore, the formulation probably did not precipitate and the bioavailability of nifedipine was not permeability-limited. By comparison, the suspension formulations resulted in considerably less exposure. The orally administered suspension was only 60% as available as the solution formulation. This may be attributable to dissolution- or solubility-limited processes. Compared to colon administration, one explanation for the slightly better exposure to nifedipine following orally administered suspension could be the difference in water-rich environments of the proximal small intestine (more fluids and surfactants—e.g., bile) compared to the colon. Although in the case of dissolution-limiting kinetics the suspension formulation may be optimized by reducing particle size (31), optimization of the suspension formulation was not attempted for this study.

Trovafloxacin (LS/HP)

The results following administration of 5 mg/kg trovafloxacin mesylate to four dogs in a crossover study are summarized in Table III. Compared to oral administration, trovafloxacin absorption from the colon was slower (T_{max}: 2.7 *vs.* 0.7 h, C_{max}: 0.18 *vs.* 0.78 μg/mL) and less complete ($AUC_{0-\infty}$: 1.03 *vs.* 3.69 μg h/mL, RBA: 25%). As shown in Fig. 2, the solubility of trovafloxacin in the native pH of the colon (~6.5) was a couple of orders of magnitude less than the dosing solution concentration. Precipitation was therefore likely following delivery to the colon. In hindsight, a formulation—perhaps with cosolvents or complexing agents—that would not precipitate at pH 6.5 would be preferred. However, this formulation was intentionally selected to replicate human intubation studies.[5] As there is no evidence that trovafloxacin permeability has a significant active component, the HP classification for this compound suggests that a formulation that does not precipitate should be well absorbed in the colon.

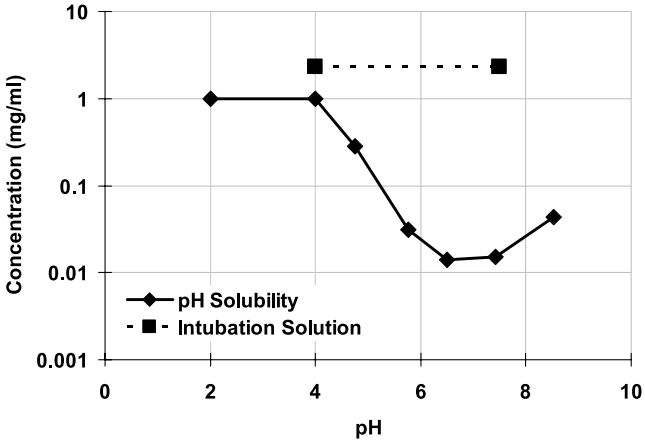

Fig. 1. Plasma nifedipine concentrations following peroral gavage and colonic administration of nifedipine to dogs (mean ± SD, n = 4; SD for oral solution omitted for clarity, are similar in magnitude to other datasets).

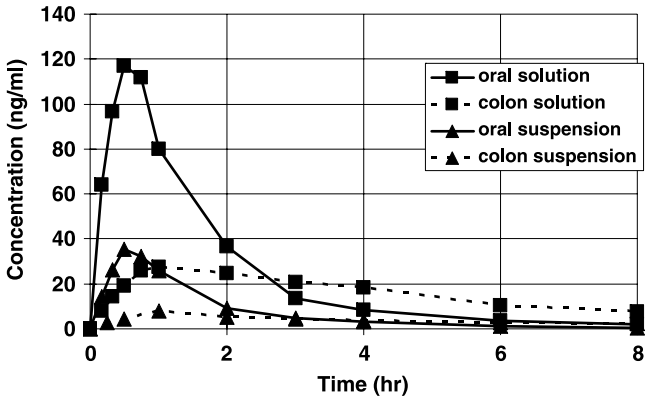

Fig. 2. Trovafloxacin pH solubility profile, showing the approximate range of gastrointestinal pH and concentration of the intubation solution.

Class 3: HS/LP

Atenolol (HS/LP)

The results following the administration of 10 mg/kg atenolol HCl to three dogs in a crossover fashion are summarized in Table III. Compared to oral administration, atenolol absorption from the colon was slower (T_{max}: 3.7 *vs.* 0.8 h, C_{max}: 1.2 *vs.* 5.2 μg/mL) and less complete ($AUC_{0-\infty}$: 13.5 *vs.* 21.8 μg h/mL). This represented an RBA of 60%. The high RBA for this "low-permeability" small, hydrophilic compound is consistent with reports on similar molecules (32).

Azithromycin (HS/LP)

The 2.5 mg/kg dose (largest dose that could reliably be administered without emesis) of azithromycin was orally administered to fasted dogs (n = 6) and a 25 mg/kg dose was administered to the colon of a separate group of fasted dogs (n = 3). The colon dose was delivered as a 5-mL bolus of a 50 mg/mL solution (Zithromax®, azithromycin for injection). Following oral azithromycin administration, serum azithromycin concentrations rapidly increased to a C_{max} of 0.177 μg/mL at 0.9 h. Compared to oral administration, colonoscopy administration resulted in slower (C_{max} of 0.16 μg/mL at 4.3 h) and markedly less complete absorption (dose-adjusted RBA 8.7%, Table III). The k_a seemed to be similar after oral and colon administrations. One possible explanation for this low RBA but similar k_a is that the colon dose rapidly moved

[5] The clinical studies are the subject of a manuscript in preparation.

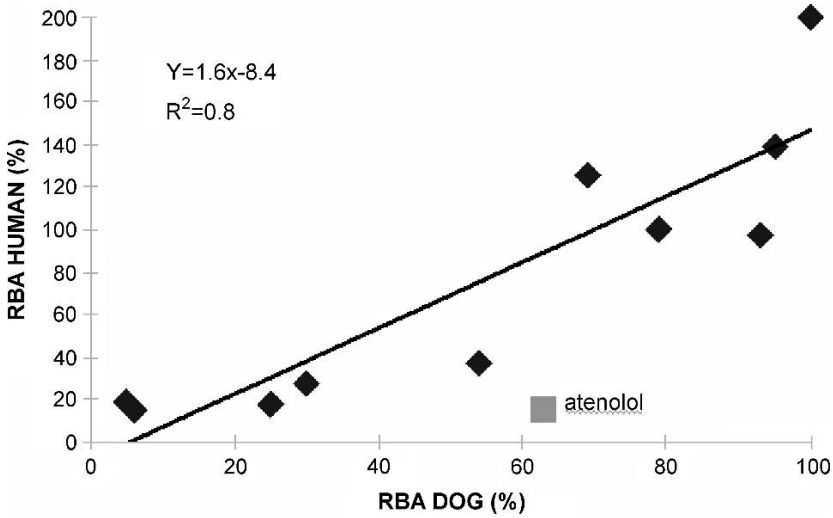

Fig. 3. Relationship between relative bioavailability in dogs and humans following oral and colon administration.

beyond a region of high permeability. This hypothesis is supported by clinical data, where administration of azithromycin to the ICJ resulted in an RBA of 81% (Sutton et al., 2006, manuscript in preparation), but only 20% when rectally administered (33).

CP-331684 (HS/LP)

The average AUC following the administration of 5 mg/kg CP-331684 to the colon of four dogs (3.21 μg h/mL) was 27% of the AUC following oral administration in a separate group (9.3, 14.6 μg h/mL, $n = 2$), Table III. Because of the large difference in AUC between the oral and colon groups (delta), this small n still provided a power of 80% certainty to detect a real delta, which was significant at the $p < 0.05$ level (34). The k_a calculated for CP-331684 following oral administration was only 0.22 h^{-1}, and log P is −2.2; passive absorption of polar, "low-permeability" compounds are largely attributed to paracellular transport. The paracellular permeability of compounds decreases as they move from the small into the large intestine, due to a greater "tightness" of the tight junctions between the epithelial cells lining the gut (35). The low RBA observed for CP-331684 was consistent with this common observation.

CP-424391 (HS/LP)

Results following the administration of 1 mg/kg CP-424391 tartrate orally and to the colon of the same five dogs are summarized in Table III. Although absorption was slightly slower following the administration to the colon (T_{max}: 1.6 *vs.* 0.6 h, C_{max}: 50 *vs.* 90 ng/mL), CP-424391 absorption was complete: $AUC_{0-\infty}$: 223 *vs.* 239 ng h/mL (RBA: 93%). These results perhaps would not be expected for "low-permeability" compounds (e.g., CP-331684 and azithromycin), which are solely absorbed by passive transport processes. However, CP-424391 seemed to be effluxed in a cell monolayer assay (Chad Stoner, personal communication), and at least some substrates for efflux transport seemed to be less effluxed in the colon than the small intestine (36). Therefore, any decrease in the passive permeability of CP-424391 in the colon was likely countered by a decrease in efflux, resulting in a net absorption similar to that after oral administration.

Class 4: LS/LP

CJ-13610 (LS/LP)

A dose of 4.5 mg/kg CJ-13610 mesylate was orally administered to four dogs. In a separate group of four dogs, 5 mg/kg CJ-13610 mesylate was also administered to the colon. As summarized in Table III, the average CJ-13610 absorption following colon administration was slower (T_{max}: 1.8 *vs.* 1.4 h, C_{max}: 0.38 *vs.* 1.13 μg/mL) and less complete ($AUC_{0-\infty}$: 2.79 *vs.* 5.89 μg h/mL). This represented a 47% RBA. As discussed for trovafloxacin, the moderately low RBA for CJ-13610 may reflect the precipitation of the administered formulation. As was the case for trovafloxacin, the CJ-13610 formulation was made at an acidic pH (reflecting the formulation administered in the clinical intubation studies),[6] where it would be in solution. However, following administration to the colon, the solution would likely be buffered—by the colon and contents—to ~6.5–7.5, resulting in a supersaturated solution that would likely result in the precipitation of CJ-13610.

DISCUSSION

This study determined the relative bioavailabilities in dogs of 11 compounds administered individually orally and to the colon. By comparing the bioavailabilities for each compound, general trends can be assessed.

Systemic bioavailabilities for six of the compounds were not limited by permeability (i.e., designated HP by BCS). Of those six, three were also not limited by solubility. Aminophylline, propranolol, and CP-409092 were all well absorbed in

[6] The clinical studies are the subject of a manuscript in preparation.

the colon, with RBA in the 70–90% range. As noted earlier, a truncated transit or residence time due to defecation had a profound effect on C_{max} and $AUC_{0-\infty}$ of aminophylline following colonic administration of aminophylline (Table III). Evidently, the truncated residence time resulted in early removal of the compound from the absorption site and a decrease in AUC.

Of the three compounds classified as BCS Class 2 (LS/HP), nifedipine solution was well absorbed from the colon, but trovafloxacin and sertraline were not. Sertraline is probably actively absorbed from the small intestine, but not from the colon. A 5-HT transporter has been identified in the intestinal epithelia (37). Because sertraline is a serotonin reuptake inhibitor, it is likely that it is also a substrate for the 5-HT transporter. Unpublished results using a Caco-2 cell monolayer model support this hypothesis (JoAnne Bennett, personal communication).

Sutton and coworkers (11) had previously reported the results of colon administration for enalapril HCl. Those data, although collected in a different laboratory (INTERx/Merck, Lawrence, KS, USA), is reviewed in this report because one of us (S.C.S.) helped develop the model at both sites, and enalapril serves as another example for an actively transported compound. Enalapril, a prodrug of the angiotensin converting enzyme inhibitor enalaprilat, is water-soluble (25 mg/mL) with a log P of 0.22 and pK_as of ~4.5, 5.5 (base) (38). The BCS classification of enalapril is controversial. Enalapril is well absorbed from the proximal small intestine by a combination of passive and active transport processes, and Kasim designated it as Class I (HS/HP). However, evidence has accumulated suggesting that the active process is via a dipeptide carrier system—a system that is probably not present in the colon (39). From a strictly passive transport definition, enalapril should be considered a Class III compound: HS/LP. After oral and colonoscopy administration of 1 mg/kg enalapril to dogs, the T_{max} values were similar (5.0 and 6.7 h). Compared to the oral route (225 ng/mL), the C_{max} values were significantly less (39.3 ng/mL), and $AUC_{0-\tau}$ were significantly less (2,370 *vs.* 735 ng h/mL, $p < 0.05$); resulting in reductions in k_a by about a third of the values after oral gavage (Table III). The RBA was about 30%. Because of the known involvement of the active transporter in the oral absorption of enalapril, it was not surprising that enalapril absorption rate was reduced after colon administration. Because most active absorptive transporters in the small intestine are not prevalent in the colon, one cannot assume that all HP compounds would also be well absorbed in the colon. The more predictive statement is: all passively absorbed (not secreted) compounds exhibiting an HP character should also be well absorbed from the colon.

Trovafloxacin has no evidence for active transport, yet the RBA following colon administration was low (25%). Because of the pH solubility profile for this compound, it is likely that the low RBA was attributable to precipitation of trovafloxacin from the supersaturated solution. This finding is consistent with the hypothesis that precipitated compound was excreted before dissolution could be completed in the relatively dry environment of the colon.

Five compounds with moderate to low permeability were also studied in the dog colon model. Of the four compounds with good solubility (BCS Class 3, HS/LP), half were well absorbed from the colon (RBA range: 70–90%). Except for atenolol, the RBA for this class of compounds seemed to be correlated with rat k_a. The relatively high RBA for the LP compound atenolol merits further discussion. Compared to human or rat, the dog absorbs small, hydrophillic compounds faster (40,41). The exceptionally good colon absorption of atenolol in the dog is probably attributable to the "leakiness" of the tight junction in that species (35). Although this is a major disadvantage of the model, the impact should be slight, as few pharmaceuticals fall into this category.

Class 4 compounds (LS/LP) are rarely successful drug candidates. The pH solubility data for CJ-13610 partially explain the low RBA; it is likely that the compound precipitated from the solution that was supersaturated by 3 orders of magnitude. A formulation—perhaps with cosolvents or complexing agents—that would not precipitate at pH 6.5 would be preferred. However, this formulation was intentionally selected to replicate planned human intubation studies. Furthermore, because the rat SPIP k_a was less than that of CP-331684, it is unlikely that even the kinetically stable solution formulation of CJ-13610 would be well absorbed from the colon.

Predictive Value for Colonic Absorption in Humans

A model is only as useful as it is predictive. During the past decade, seven of the eight Pfizer compounds had been examined in clinical intubation studies. The colon absorption of nifedipine has been reported in the literature by Bode and coworkers (20). These studies were carried out in groups of six normal volunteers, following informed consent. The compound was administered orally and via enteronasal tube into the ICJ or colon in formulations similar to those administered to dogs. Serial blood samples were collected and the concentration of administered compound in plasma/sera was determined by a validated assay. Complete details of these studies are the subject of another report. The bioavailability of each compound after ICJ or colon administration relative to their oral or duodenal administration (RBA) was compared to the values obtained in the dog (Fig. 3). Also included in Fig. 3 are the results for propranolol (30), aminophylline (24), and atenolol (42) (atenolol was not included in the correlation calculations). The RBA in dog correlated well with that in the clinic (Sutton et al., 2006, manuscript in preparation). The correlation coefficient (r^2 = 0.92) suggests that the dog model predicts the colon permeability of these compounds reasonably well.

CONCLUSION

Although the colonoscopy procedure seems straightforward, the physiological, pharmaceutical, and physical chemical processes involved (transit time, solubility, dose, formulation, absorption rate/mechanism) are complex. For example, the method for increasing solubility of poorly soluble agents could affect the outcome. We observed one tightly bound complex between a compound and cyclodextrin, which resulted in an unexpected decrease in bioavailability (data not shown). Supersaturation could also lead to uncontrolled crystallization and aggregation, potentially

resulting in larger particles than the starting material. The larger particles could dissolve even more slowly than the starting material, delaying absorption, which in turn may be incomplete due to excretion.

While appreciating the complexity of the problem, this work supported the dog colonoscopy model as a simple method to rapidly predict relative colonic absorption of a CR candidate in humans. As no surgery or additional maintenance was needed, the method is readily applicable to every facility that houses dogs. The anticipated wide applicability of the model should greatly aid in the optimal allocation of resources to those CR formulation candidates where success (i.e., acceptable colonic absorption) is most likely. It is therefore proposed that the dog colon model be used as a surrogate for human intubation studies when the CR candidate falls in the BCS Classes 2 (LS/HP), Class 3 (HS/LP), and Class 4 (LS/LP). However, for compounds in Class 1 (HS/HP) the authors have always found that colon absorption was high, and suggest that the CR development of these compounds do not require the confirmation of either dog colon or human intubation studies.

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GLOSSARY

AC: ascending colon
AM: arithmetic mean
AMT: Asymmetric membrane technology tablet
$AUC_{0-\infty}$: area under the plasma concentration–time curve from 0 h and extrapolated to infinity
BCS: Biopharmaceutical Classification Scheme
BLQ: below LLOQ
C_{max}: first occurrence of the maximum plasma concentration
C_{min}: the minimum plasma concentration during a dosing interval
CR: controlled release formulation
CV: coefficient of variation (percentage), given by: 100 * (SD/AM)
FIH: first-in-human studies
GI: gastrointestinal
GIT: gastrointestinal tract
GM: geometric mean
HP: high permeability
HS: high solubility
ICJ: ileal–cecal junction
IR: immediate release formulation
IS: internal standard
k_a: absorption rate constant
k_{el}: elimination rate constant—used to extrapolate AUC from the last time point (C_{LTP}) to infinity with the following Eqn: $AUC_{LTP-\infty} = C_{LTP}/k_{el}$
LLOQ: lower limit of quantification
LP: low permeability
LS: low solubility
P_{app}*: apparent permeability*
PGRD: Pfizer Global Research & Development
RBA: bioavailability $AUC_{0-\infty}$ *relative to an orally administered dose*
SD: standard deviation
SE: standard error
SPIP: (rat) single pass intestinal perfusion
TC: transverse colon
T_{max}*: time of* C_{max}

REFERENCES

1. L. R. Johnson. *Physiology of the Gastrointestinal Tract. vol. I*, Raven Press, New York, 1994.
2. P. Bieck. *Colonic Absorption and Metabolism*, Marcel Dekker, NY, 1993.
3. N. Rietbrock. *Drug Absorption from Different Regions of the Human GI Tract: Methods of Investigation and Results*, Vieweg and Sohn, Braunschweig/Wiesbaden, 1987.
4. B. Henry, K. Horspool, M. Humphrey, and J. Nuttall. Absorption of peptide-like drugs, *Formulations of poorly-available drugs for oral administration*, APGI & Swedish Academy of Pharmaceutical Sciences, Editions de Sante, Paris, 1996, pp. 195–198.
5. R. Oberle and H. Das. Variability in gastric pH and delayed gastric emptying in Yucatan miniature pigs. *Pharm. Res.* **11**: 592–594 (1994).
6. J. Kim, R. Oberle, D. Krummel, J. Dressman, and D. Fleisher. Absorption of ACE inhibitors from small intestine and colon. *J. Pharm. Sci.* **83**:1350–1356 (1994).
7. J. Tibbits. Issues related to the use of canines in toxicologic pathology—issues with pharmacokinetics and metabolism. *Toxicol. Pathol.* **31**(Suppl):17–24 (2003).
8. J. Fix, K. Engle, P. Porter, P. Leppert, S. Selk, C. Gardner, and J. Alexander. Acylcarnitines: drug absorption-enhancing agents in the gastrointestinal tract. *Am. J. Physiol.* **250**:G332–G340 (1986).
9. L. Meunier, J. Kissinger, J. Marcello, A. Nichols, and P. Smith. A chronic access port model for direct delivery of drugs into the intestine of conscious dogs. *Lab. Animal. Sci.* **43**:466–470 (1993).
10. N. J. Clear, A. Milton, M. Humphrey, B. T. Henry, M. Wulff, D. J. Nichols, R. J. Anziano, and I. Wilding. Evaluation of the Intelisite capsule to deliver theophylline and frusemide tablets to the small intestine and colon. *Eur. J. Pharm. Sci.* **13**:375–384 (2001).
11. S. C. Sutton, K. Engle, and M. Cortese. Conscious dog model for the study of colon absorption and dosage formulation performance in the colon. *Pharm. Res.* **8**:S159 (1991).
12. FDA. Guidance for Industry: Waiver of *in Vivo* Bioavailability and Bioequivalence Studies for Immediate Release Solid Oral Dosage Forms Containing Certain Active Moieties/Active Ingredients Based on a Biopharmaceutics Classification System., CDER, Rockville, 2000.
13. M. A. Bach and M. Cambria. Growth hormone releasing peptides: growth studies. *Curr. Opin. Endocrinol. Diabetes* **6**: 100–105 (1999).
14. J. T. MacAndrew, S. S. Ellery, M. A. Parry, L. C. Pan, and S. C. Black. Efficacy of a growth hormone-releasing peptide mimetic in cardiac ischemia/reperfusion injury. *Eur. J. Pharmacol.* **432**:195–202 (2001).

15. T. Mano, R. W. Stevens, K. Ando, K. Nakao, Y. Okumura, M. Sakakibara, T. Okumura, K. Miyamoto, and T. Tamura. CJ-13610: Design of an Orally Active and Safe 5-LO Inhibitor, 22nd Symp. Med. Chem., Shizuoka, Japan, 2002, pp. 12–19.
16. *Merck Index*, Merck Research Laboratories, Whitehouse Station, 1997.
17. E. F. Fiese. General pharmaceutics—the new physical pharmacy. *J. Pharm. Sci.* **92**:1331–1342 (2003).
18. S. C. Sutton, M. T. S. Rinaldi, J. M. McCarthy, and K. E. Vukovinsky. A statistical method for the determination of absorption rate constant estimated using the rat single pass intestinal perfusion model and multiple linear regression. *J. Pharm. Sci.* **91**:1046–1053 (2002).
19. S. C. Sutton, M. T. S. Rinaldi, and K. E. Vukovinsky. Comparison of the gravimetric, phenol red and 14C-PEG-3350 methods to determine water absorption in the rat single pass intestinal perfusion model. *AAPS Pharm. Sci.* **3**:article 25 (2001).
20. H. Bode, E. Brendel, G. Ahr, U. Fuhr, and S. Harder. Investigation of nifedipine absorption in different regions of the human git after simultaneous administration of 13C and 12C nifedipine. *Eur. J. Clin. Pharmacol.* **50**:195–201 (1996).
21. S. C. Sutton, L. Fournier, and K. Norton. Dog colonoscopy model for predicting human colon absorption. *Pharm. Res.* **12**: S221 (1995).
22. C. Hop, Z. Wang, Q. Chen, and G. Kwei. Plasma-pooling methods to increase throughput for *in vivo* pharmacokinetic screening. *J. Pharm. Sci.* **87**:901–903 (1998).
23. R. Teng, T. G. Tensfeldt, T. E. Liston, and G. Foulds. Determination of trovafloxacin, a new quinolone antibiotic, in biological samples by reversed-phase high-performance liquid chromatography. *J. Chromatogr. B* **675**:53–59 (1996).
24. A. H. Staib, D. Loew, S. Harder, E. H. Graul, and R. Pfab. Measurement of theophylline absorption from different regions of the gastro-intestinal tract using a remote controlled drug delivery device. *Eur. J. Clin. Pharmacol.* **30**:691–697 (1986).
25. C. J. Fu and W. D. Mason. A simplified method for determination of nifedipine in human plasma by HPLC. *Anal. Lett.* **22**: 2985–3002 (1989).
26. R. M. Shepard, G. S. Duthu, R. A. Ferraina, and M. A. Mullins. HPLC assay with EC detection of azithromycin in serum and tissues. *J. Chromatogr., B, Biomed. Appl.* **565**:321–337 (1991).
27. S. Yee, Z. Lin, M. Gunduz, S. Jaw-Tsai, and F. R. Nelson. Effect of P-glycoprotein on the oral absorption and brain penetration of CP-409,092 in rats and mice. *Drug Metab. Rev.* **32**:194 (2000).
28. P. A. Carpino, B. A. Lefker, S. M. Toler, L. C. Pan, J. Hadcock, P. Dasilva-Jardine, J. Pettersen, and D. Thompson. Short- and long-acting growth hormone secretagogues: discovery and SAR of capromorelin tartrate (CP-424391-18, American Chemical Society National Meeting, Chicago, IL, 2001.
29. D. Taylor, R. Grundy, and B. Loveday. Chronic dog intestinal loop model for studying drug absorption as exemplified by beta-adrenoreceptor blocking agents, atenolol and propranolol. *J. Pharm. Sci.* **70**:516–521 (1981).
30. A. Buch and W. Barr. Absorption of propranolol in humans following oral, jejunal, and ileal administration. *Pharm. Res.* **15**:953–957 (1998).
31. K. C. Waterman and S. C. Sutton. A computational model for particle size influence on drug absorption during controlled-release colonic delivery. *J. Control. Release* **86**:293–304 (2003).
32. J. Dressman and K. Yamada. Animal models for oral drug absorption. In P. Welling and F. Tse (eds.), *Pharmaceutical Bioequivalence*, Dekker, New York, 1991, pp. 235–266.
33. D. Luke, P. Going, G. Foulds, and G. Melnik. Bioavailability of azithromycin when delivered as a rectal solution to healthy subjects, *The Third International Conference on the Mac rolides, Azalides and Streptogramins*, Lisbon, Portugal, 1996, Abstract #4.25.
34. R. Sokal and F. Rohlf. *Biometry*, WH Freeman, New York, 1981.
35. E. L. LeCluyse and S. C. Sutton. *In vitro* models for selection of development candidates. Permeability studies to define mechanisms of absorption enhancement. *Adv. Drug Deliv. Rev.* **23**: 163–183 (1997).
36. V. D. Makhey, A. Guo, D. A. Norris, P. Hu, J. Yan, and P. J. Sinko. Characterization of the regional intestinal kinetics of drug efflux in rat and human intestine and in Caco-2 cells. *Pharm. Res.* **15**:1160–1167 (1998).
37. P. Wade, J. Chen, B. Jaffe, I. Kassem, R. Blakely, and M. Gershon. Localization and function of a 5-HT transporter in crypt epithelia of the gastrointestinal tract. *J. Neurosci.* **16**: 2352–2364 (1996).
38. N. H. Kasim, M. Whitehouse, C. Ramachandran, M. Bermejo, H. Lennernäs, A. S. Hussain, H. E. Junginger, S. A. Stavchansky, K. K. Midha, V. P. Shah, and G. L. Amidon. Molecular properties of WHO essential drugs and provisional biopharmaceutical classification. *Mol. Pharm.* **1**:85–96 (2003).
39. D. Friedman and G. L. Amidon. Passive and carrier mediated intestinal absorption components of two ACE inhibitor prodrugs in rats: enalapril and fosinopril. *Pharm. Res.* **6**:1043–1047 (1989).
40. W. L. Chiou, H. Jeong, S. Chung, and W. TC. Evaluation of using the dog as an animal model to study the fraction of oral dose absorbed of 43 drugs in humans. *Pharm. Res.* **17**:135–140 (2000).
41. W. L. Chiou and A. Barve. Linear correlation of the fraction of oral dose absorbed of 64 drugs between humans and rats. *Pharm. Res.* **15**:1792–1795 (1998).
42. S. A. Riley, M. Kim, F. Sutcliffe, M. Rowland, and L. A. Turnberg. Absorption of polar drugs following caecal instillation in healthy volunteers. *Aliment. Pharmacol. Ther.* **6**:701–706 (1992).
43. I. Moriguchi, S. Hirono, I. Nakagome, and H. Hirano. Comparison of reliability of log *P* values for drugs calculated by several methods. *Chem. Pharm. Bull.* **42**:976–978 (1994).
44. C. Hoyo-Vadillo, G. Castaneda-Hernandez, J. E. Herrera, J. Vidal-Garate, L. A. Salazar, A. Moreno-Ramos, F. Chavez, L. Tena, and E. Hong. Pharmacokinetics of Oral Nifedipine: Relevance of the Distribution Phase. *J. Clin. Pharmacol.* **29**: 251–256 (1989).
45. M. L. Martins, M. A. Pierossi, L. A. Moraes, W. Ribeiro, E. Abbib, G. B. Mendes, A. Poli, G. De Nucci, and M. N. Muscara. Comparative bioavailability of two atenolol tablet formulations in healthy male volunteers after a single dose administration. *Int. J. Clin. Pharmacol. Ther.* **35**:324–328 (1997).