Mass Balance Approaches for Estimating the Intestinal Absorption and Metabolism of Peptides and Analogues: Theoretical Development and Applications

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Received April 14, 1992; accepted August 22, 1992

A theoretical analysis for estimating the extent of intestinal peptide and peptide analogue absorption was developed on the basis of a mass balance approach that incorporates convection, permeability, and reaction. The macroscopic mass balance analysis (MMBA) was extended to include chemical and enzymatic degradation. A microscopic mass balance analysis, a numerical approach, was also developed and the results compared to the MMBA. The mass balance equations for the fraction of a drug absorbed and reacted in the tube were derived from the general steady state mass balance in a tube:

$$\frac{dM}{dz} = \{[(2/R)(P_w + k_r)]CV_L\}/v_z,$$

where M is mass, z is the length of the tube, R is the tube radius, P_w is the intestinal wall permeability, k_r is the reaction rate constant, C is the concentration of drug in the volume element over which the mass balance is taken, V_L is the volume of the tube, and v_z is the axial velocity of drug. The theory was first applied to the oral absorption of two tripeptide analogues, cefaclor (CCL) and cefatrizine (CZN), which degrade and dimerize in the intestine. Simulations using the mass balance equations, the experimental absorption parameters, and the literature stability rate constants yielded a mean estimated extent of CCL (250-mg dose) and CZN (1000-mg dose) absorption of 89 and 51%, respectively, which was similar to the mean extent of absorption reported in humans (90 and 50%). It was proposed previously that 15% of the CCL dose spontaneously degraded systemically; however, our simulations suggest that significant CCL degradation occurs (8 to 17%) presystemically in the intestinal lumen. Insulin (M_r = 5700), which is metabolized in the intestine primarily by α-chymotrypsin, was chosen for the second application of theory. The simulations show that the intestinal absorption of insulin is approximately 1% of the administered dose. Further, the extent of insulin oral absorption may not exceed 2% even if effective enzyme inhibitors are dosed concurrently since simulations show that insulin absorption is permeability limited. The steady-state macroscopic and microscopic simulation results were comparable and, for the antibiotics, were similar to published clinical results. Therefore, both approaches are useful for estimating the extent of oral peptide absorption and intestinal reaction from *in vitro* and *in situ* results.

KEY WORDS: extent of absorption; mass balance; intestinal metabolism; cefaclor; cefatrizine; chymotrypsin; insulin; peptides.

INTRODUCTION

The oral delivery of peptides and analogues is compromised by chemical and proteolytic instability as well as by intestinal membrane transport limitations. Small peptides (three amino acid residues or smaller) are absorbed primarily by a carrier-mediated absorption mechanism, whereas larger peptides and small proteins are absorbed by paracellular and endocytotic mechanisms (1). The enzymatic metabolism and chemical instability of peptides and peptide drug analogues pose a formidable challenge to oral peptide delivery. GI proteases begin the digestion of orally delivered peptides and proteins by hydrolyzing peptide bonds, producing smaller peptides and amino acids that are then absorbed into the enterocyte. Amino acids and di- and tri-peptides are absorbed intact, whereas 90% of peptides with more than three amino acid residues are metabolized by GI digestive enzymes (2). Cytosolic metabolism accounts for 85 to 95% of the digestion of dipeptides, whereas tripeptides are metabolized by both the cytosolic and the brush border enzymes.

Since gastric proteolysis accounts for only approximately 15% of the metabolism of dietary protein, the primary role of the stomach in protein metabolism is that it acts as a reservoir—metering the entry of protein into the small intestine to ensure thorough mixing with pancreatic enzymes (3). Presystemic peptide metabolism occurs in the intestinal lumen, brush border, and enterocyte. Based on the physiological role of each of these enzyme systems in the digestion process, the pancreatic serine proteases (lumenal enzymes) represent the first significant barrier to systemic peptide delivery. Since the lumenal enzymes traverse the small intestine at the same velocity as the intestinal contents, the mass balance approach is well suited for modeling this physiological system. In a recent publication, Sinko *et al.* (4) developed a macroscopic mass balance model for estimating the extent of oral absorption for compounds absorbed by carrier-mediated and/or passive absorption mechanisms. In that report, *in situ* absorption parameters were successfully correlated with extent of absorption results in humans. In this report, the macroscopic theory is extended to include chemical and enzymatic degradation. Moreover, a microscopic (numerical) approach is developed and the results are compared to those of the macroscopic approach.

THEORETICAL

A general mass balance for a drug in solution in an arbitrary differential fluid element can be written

$$\frac{\partial C}{\partial t} + v_z \cdot \nabla C = \nabla \cdot (D \nabla C) - R_L \qquad (1)$$

where C is the concentration of drug, t is time, v_z is the axial (longitudinal) velocity of fluid and drug, D represents both the axial and the radial molecular dispersion coefficients, and R_L is the rate of loss of drug due to absorption and chemical and/or enzymatic degradation. Assuming cylindri-

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0724-8741/93/0200-0271$07.00/0

cal intestinal geometry, constant temperature and pressure, constant molecular dispersion coefficients, dilute drug concentrations, and constant mass density, Eq. (1) can be written as (5)

$$\frac{\partial C}{\partial t} + v_z \frac{\partial C}{\partial z} = D_z \frac{\partial^2 C}{\partial z^2} + D_r\left(\frac{\partial^2 C}{\partial r^2} + \frac{1}{r}\frac{\partial C}{\partial r}\right) - R_L \quad (2)$$

where D_z is the axial dispersion coefficient and D_r is the radial dispersion coefficient. An ideal flow model, the plug flow model, is used in this analysis. Plug flow is characterized by complete radial mixing but no axial mixing, therefore, plug flow volume elements have identical velocities and residence times. While idealized flow models may not be physiologically realistic, they have shown utility in previous theoretical-experimental correlations (4). For the plug flow model, D_r is assumed large, thereby making the radial concentration gradient unimportant. Furthermore, the axial dispersion coefficient, D_z, is 0 and drops out of Eq. (2). Therefore, for the plug flow model Eq. (2) becomes

$$-\frac{\partial C}{\partial t} = v_z \frac{\partial C}{\partial z} + k_a C + k_r C \quad (3)$$

for the plug flow model, where k_a and k_r are the absorption and degradation rate constants, respectively. As seen in Eq. (3), drug loss in the tube occurs by the three processes occurring simultaneously: absorption, convection, and reaction.

On a mass basis Eq. (3) becomes

$$-\frac{\partial M}{\partial t} = v_z \frac{\partial M}{\partial z} + (k_a + k_r) C V_L \quad (4)$$

and, assuming steady state, becomes

$$\frac{-dM}{dz} = \frac{(k_a + k_r) C V_L}{v_z} \quad (5)$$

Macroscopic and microscopic approaches are used to solve Eq. (5). Solving the differential equation using the macroscopic approach is difficult if nonlinear processes such as dissolution rate, nonpassive permeability, and intestinal metabolism are included in the model. Therefore, the nonpassive permeability term is treated using a concentration-averaged permeability known as the *mean permeability*. The mean permeability was defined previously (6) as

$$\overline{P^*_w} = \frac{\int_{C_o}^{0} P^*_w \, dC}{\int_{C_o}^{0} dC} = P^*_m + P^*_c \frac{K_m}{C_o} \ln\left(1 + \frac{C_o}{K_m}\right)$$

where C_o is the inlet concentration, K_m is the Michaelis constant, and P^*_w is the dimensionless wall permeability. For the microscopic approach the nonlinearity is solved numerically.

Macroscopic Approach

Substituting the following into Eq. (5)

$$k_a = 2P_w/R$$
$$P^*_w = P_w R/D_{aq}$$
$$z^* = z/L$$
$$V_L = \pi R^2 L$$
$$v_z = Q/\pi R^2$$
$$t_{res} = V_L/Q$$

separating the variables, and rearranging, the fraction of drug lost from the intestine due to absorption and reaction can be written as

$$F_L = 1 - \frac{C_m}{C_o} = \left(\frac{2\pi D_{aq} L}{Q} P^*_w + k_r \frac{V_L}{Q}\right) \int_0^1 C^*_w \, dz^* \quad (6)$$

When the parameters, An (absorption number) and Da (Damköhler number), are substituted into Eq. (6), the general expression for the simultaneous absorption, bulk fluid flow, and reaction of drugs in the tube becomes

$$F_L = (2An + Da) \int_0^1 C^*_w \, dz^* \quad (7)$$

where

$$An = \frac{L}{R}\frac{P_e}{v_z} \quad (8)$$

and

$$Da = k_r \frac{V_L}{Q} = k_r \overline{t_{res}} \quad (9)$$

The drug concentration profile in the tube, $\int_0^1 C^*_w \, dz^*$, for the plug flow model (4) is

$$C^*_w = e^{-(2An + Da)z^*} \quad (10)$$

therefore, the fraction of drug lost in the tube for the plug flow model becomes

$$F_L = 1 - e^{-(2An + Da)} \quad (11)$$

The fraction of the dose that is absorbed from the tube is

$$F_A = \frac{2An}{2An + Da}\left[1 - e^{-(2An + Da)}\right] \quad (12)$$

whereas the fraction of the dose that is degraded in the tube is

$$F_R = \frac{Da}{2An + Da}\left[1 - e^{-(2An + Da)}\right] \quad (13)$$

Microscopic Approach

While the results of the macroscopic approach yield analytical solutions, the microscopic simulations are performed using numerical routines so that nonlinear absorption and reaction can be evaluated. The following differential equation is written from Eq. (5):

$$\frac{-dC^*}{dz^*} = (2An + Da) C^* \quad (14)$$

where An equals

$$An = Gz^{PF}\left(\frac{P^*_c}{1 + \frac{C^*C_o}{K_m}} + P^*_m\right) \quad (15)$$

for a carrier-mediated plus passive intestinal absorption mechanism. If absorption occurs by a passive mechanism, *An* becomes

$$An = Gz^{PF} P^*_m \quad (16)$$

The reaction term, *Da*, can be written to fit the needs of the particular application. For the applications of this analysis it is convenient to write *Da* in two ways,

$$Da = (k_1 + k_2 C^*C_o)\, t_{res} \quad (17)$$

$$Da = \left(\frac{V_{max}}{K_m + C^*C_o}\right) t_{res} \quad (18)$$

ACSL (Advanced Continuous Simulation Language, Mitchell & Gauthier Associates, Concord, MA) was used to perform the microscopic simulations. ACSL simulations were performed using a fixed step, fourth-order Runge–Kutta numerical integration method with the following boundary condition:

$$z^* = 0, \qquad C^* = 1$$

For all simulations the human small intestinal residence time is taken to be 3 hr (7). The Graetz number for the plug flow model (4) is 1.27 and the volume given with the dose is 200 mL (also taken to be the volume in the intestine). Mass transport and reaction parameters are taken from *in situ* or *in vitro* results. The absorption parameters, previously found to correlate well with the extent of absorption results in humans, are used in these simulations (4). The intrinsic intestinal transport parameters, as determined in the rat small intestine, are given in Table I.

RESULTS AND DISCUSSION

Application 1: Tripeptide Analogues

Cefaclor (CCL)

CCL is well absorbed in humans (8,9), with absorption occurring rapidly. Peak concentrations are observed approximately 1 hr after oral dosing in fasted subjects. CCL metabolism in the liver is minimal (8) and elimination is rapid, with 60 to 85% of the dose excreted unchanged in the urine. Eighty-four percent of the fraction excreted into the urine occurs within the first 2 hr (10). CCL is unstable in biological fluids (11). The initial intestinal concentrations of cefaclor are estimated by dividing the dose given by the volume taken with the dose. Therefore, if 250 mg of cefaclor is given with 200 mL of water, the initial intestinal CCL concentration is 3.2 m*M*. Since the absorption K_m is 16 m*M* and the initial dose-concentration is below the K_m, linear absorption behavior is expected. Linearity is confirmed in human studies on the basis of the dose proportionality observed in the reported C_{max} and AUCs. Nakashima *et al.* (11) studied the stability of CCL and concluded that two processes were responsible for CCL degradation *in vivo*, first-order degradation by intramolecular nucleophilic attack and, at higher concentrations, a dimerization reaction.

Table I. Intestinal Transport Parameters of Orally Absorbed Cephalosporins, reported as value (SD)

Drug	P^*_c	K_m (m*M*)	P^*_m	Ref. no.
Cefatrizine	1.3 (0.1)	0.6 (0.2)	0.2 (0.03)	17
Cefaclor	1.3 (0.1)	16 (3.6)	ND0[a]	17

[a] Not different from zero.

Simulations were performed using *in situ* absorption parameters from rats (Table I) and the *in vivo* stability rate constants (11) reported in the literature. Three sets of simulations were performed. *Simulation 1:* $k_1 = 0.0941\ hr^{-1}$, $k_2 = 0.004\ mM^{-1}\ hr^{-1}$, microscopic approach [Eq. (14)]. *Simulation 2:* $k_1 = 0.0941\ hr^{-1}$, $k_2 = 0$, microscopic approach [Eq. (14)]. *Simulation 3:* $k_1 = 0.0941\ hr^{-1}$, $k_2 = 0$, macroscopic approach [Eqs. (11)–(13)]. k_1 is the stability rate constant and k_2 is the dimerization rate constant.

The results of the CCL simulations are given in Table II. The fraction of the 250-mg dose absorbed was calculated to be 89, 89, and 88% for simulations 1, 2, and 3, respectively. All of the simulation results compare favorably to the reported value in humans of 90% (8). The estimated fraction of the CCL dose degraded in the intestine was calculated to be 7.7, 7.7, and 8.5%, respectively. Although the elimination of CCL (8) has been attributed to rapid renal elimination (85%) and spontaneous systemic degradation (15%), these simulations suggest that approximately 50% of CCL degradation occurs presystemically. Since CCL is an effective antibiotic at low doses, nonlinear absorption behavior and chemical instability are not clinically significant. In the next example, however, the absorption K_m of cefatrizine is low enough to affect the rate and extent of absorption.

Cefatrizine (CZN)

Pfeffer *et al.* (12) studied the human intravenous pharmacokinetics and absolute oral bioavailability of CZN in human subjects. Three doses (250, 500, and 1000 mg) were given in a crossover fashion. Intravenous pharmacokinetics were dose linear. The oral bioavailability at the three doses was 75, 75, and 50%, respectively. Other pharmacokinetic parameters such as C_{max} and t_{max} showed dose-dependent changes consistent with nonlinear intestinal absorption. The decrease in oral bioavailability with increasing dose is consistent with the low K_m determined for CZN in rats. CZN, like CCL, is unstable in intestinal fluid, with first-order degradation and, at higher doses, second-order dimerization occurring.

When 1000 mg of CZN is given with 200 mL of water, the initial intestinal concentration is 11 m*M*. Since the K_m for absorption for CZN is much lower, at 0.6 m*M*, zero-order absorption behavior is expected. Moreover, at initial dose-concentrations above 10 m*M*, significant dimerization reactions are possible (11). Three simulations were performed for the 1000-mg dose. *Simulation 1:* $k_1 = 0.0824\ hr^{-1}$, $k_2 = 0.00935\ mM^{-1}\ hr^{-1}$, microscopic approach [Eq. (14)]. *Simulation 2:* $k_1 = 0.0824\ hr^{-1}$, $k_2 = 0$, microscopic approach [Eq. (14)]. *Simulation 3:* $k_1 = 0.0824\ hr^{-1}$, $k_2 = 0$, macroscopic approach [Eqs. (11)–(13)].

Table II. Cefaclor and Cefatrizine Simulation Results[a]

Drug	Dose (mg)	Microscopic with dimerization FR (%)	Microscopic with dimerization FA (%)	Microscopic without dimerization FR (%)	Microscopic without dimerization FA (%)	Macroscopic FR (%)	Macroscopic FA (%)	Reported, FA (%)
Cefaclor	250	7.7	89	7.7	89	8.5	88	90
Cefatrizine	1000	17	54	13	51	16	48	50

[a] FA, fraction of dose absorbed; FR, fraction of dose reacted.

The results of the simulations are given in Table II. The calculated extents of absorption for the 1000-mg dose are 54, 51, and 48% for the three simulations, respectively. The reported fraction of CZN absorbed in humans at a 1000-mg dose is 50% (12). The reported literature value in humans compares favorably to the simulated values. The extent of degradation was significant at 17, 13, and 16% for the three simulations, respectively.

The model for the wall permeability used throughout the analysis is a combination of carrier-mediated and passive absorption mechanisms:

$$P^*_w = \frac{J^*_{max}}{K_m + C_w} + P^*_m$$

At concentrations that are well below the K_m, the Michaelis–Menten term becomes the carrier permeability, $P^*_c = J^*_{max}/K_m$. As shown in Table I, the carrier permeability for CCL and CZN are equal, therefore, at doses well below their respective K_m's the antibiotics should be equally well absorbed. However, the effect of dose on the permeability (and, thus, the absorption rate) of CZN is considerable. Since the K_m of CZN is very low, the mean P^*_w for CZN is only 0.3 at the 1000-mg dose, whereas the mean wall permeability of CCL is 1.1 at the 250-mg dose. The mean permeability of CZN at 1000 mg is nominally larger than the passive permeability, indicating slow absorption and an increased likelihood of intestinal degradation. The current analysis and simulations suggest that the lower extent of CZN absorption is attributable (1) to slower absorption due to CZN's low absorption Michaelis constant and (2) to significant intestinal degradation.

Application 2: A Larger Polypeptide, Insulin

Human insulin (M_r = 5700) is a polypeptide hormone comprised of two peptide chains with a total of 51 amino acid residues. Donovan *et al.* (13) demonstrated in rats that the absorption of molecules larger than 1300 daltons was limited but constant at approximately 2%. Therefore, based on molecular size alone, the absorption of insulin is expected to be permeability limited. The oral absorption of insulin is further compromised by intestinal metabolism from the pancreatic serine proteases, α-chymotrypsin and trypsin (14).

For the simulations (Table III), chemical instability and brush border metabolism are considered negligible. α-Chymotrypsin and trypsin kinetic parameters (pH 8, 37°C) and intestinal permeabilities are taken from the literature (14,15). Intestinal permeabilities are made dimensionless by multiplying P_e by R/D_{aq}, where R is the radius of the intestine (1 cm for the rat) and D_{aq} is the aqueous diffusion coefficient. The aqueous diffusion coefficient of insulin is calculated using the Hayduk–Laudie method of partial molal (Schroeder) volumes. D_{aq} for insulin is calculated to be 1.2×10^{-6} cm^2/sec. The mean first-order degradation rate constant for the macroscopic simulations takes the form

$$\overline{k_r} = \frac{V_{max}}{C_E} \ln\left(1 + \frac{C_E}{K_m^E}\right)$$

for intestinal metabolic reactions, where C_E is the concentration of enzyme, K_m^E is the Michaelis constant of the enzyme, and V_{max} is the maximal velocity. α-Chymotrypsin levels in the duodenum of humans have been reported to be 1.3×10^{-6} M (16).

The upper limit of insulin oral absorption can be calculated if it is assumed that insulin is stable in the GI tract (Da = 0). On this basis, the maximal extent of insulin absorption in the duodenum is 1.6%, whereas it is calculated to be 13% in the ileum. The estimated absorption of insulin from the duodenum is consistent with the value of 2% reported by Donovan *et al.* (13) for PEG absorption. The ileal value cannot be verified since literature data for the ileal absorption of insulin are lacking. Simulations were performed using the parameters in Table IV and estimates of the extent of absorption and reaction were obtained. The results of the macroscopic and microscopic simulations for the duodenal and ileal segments and the effects of α-chymotrypsin, trypsin, and a combination of both enzymes on the extent of insulin absorption are given in Table IV. With one exception, the calculated extent of insulin absorption from the intestine was less than or equal to 1.20%. Most of the insulin was degraded rather than absorbed in all of the simulations. Although degradation is a limiting factor, the extremely low intestinal per-

Table III. Insulin Simulation Parameters

	Parameter/value	Ref. no.
Absorption		15
Duodenum	P^*_e = 0.0063	
Ileum	P^*_e = 0.055	
Metabolism		14
α-Chymotrypsin	$V_{max} = 6.48 \times 10^{-6}$ M/min	
	K_m = 0.100 mM	
Trypsin	$V_{max} = 0.75 \times 10^{-6}$ M/min	
	K_m = 0.099 mM	
Scaling	t_{res} = 3 hr	7
	Gz^{PF} = 1.27	4
	Volume = 200 mL	—

Table IV. Insulin Simulation Results[a]

	Microscopic		Macroscopic	
	FA (%)	FR (%)	FA (%)	FR (%)
Duodenum				
α-Chymotrypsin	0.2	92	0.1	99.9
Trypsin			0.9	74
Both			0.1	99.9
Ileum				
α-Chymotrypsin	1.7	93	1.2	99
Trypsin			7.3	70
Both			1.1	99

[a] FA, fraction of dose absorbed; FR, fraction of dose reacted.

meability of insulin is the significant obstacle to the oral delivery of insulin.

ACKNOWLEDGMENTS

Support from NIH Grant GM37188 (University of Michigan) and NIH BRSG 4-23444 (Rutgers University) is gratefully acknowledged.

NOMENCLATURE

C	Concentration
t	Time
v_z	Axial (convective) velocity
D	Molecular dispersion coefficient
R_L	Rate of loss of drug in tube
z	Axial dimension
r	Radial dimension
D_r	Radial dispersion coefficient
D_z	Axial dispersion coefficient
k_a	Absorption rate constant
k_r	Reaction rate constant
M	Drug mass
R	Radius of tube
P_w	Wall permeability
t_{res}	Mean residence time in the tube
P_e	Effective drug permeability
Da	Damköhler number
An	Absorption number
D_{aq}	Aqueous diffusion coefficient
L	Length of the tube
V_L	Volume of the tube
Q	Volumetric flow rate
F_L	Fraction of drug lost in the tube due to absorption and reaction
F_A	Fraction of drug absorbed in the tube
F_R	Fraction of drug reacted in the tube
C_o	Inlet drug concentration
C_m	Outlet (cup mixing) concentration
C_w	Concentration of the drug at the tube wall
V_{max}	Maximal velocity
K_m^E	Enzymatic Michaelis constant
C_E	Concentration of enzyme

Superscript

*	Denotes a dimensionless quantity

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