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Research Article

Predicting Fraction Dose Absorbed in Humans Using a Macroscopic Mass Balance Approach

Patrick J. Sinko,[1,2] Glen D. Leesman,[1,2] and Gordon L. Amidon[1,3]

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A theoretical approach for estimating fraction dose absorbed in humans has been developed based on a macroscopic mass balance that incorporates membrane permeability and solubility considerations. The macroscopic mass balance approach (MMBA) is a flow model approach that utilizes fundamental mass transfer theory for estimating the extent of absorption for passively as well as nonpassively absorbed drugs. The mass balance on a tube with steady input and a wall flux of $J_w = P_w C_b$ results in the following expression for fraction dose absorbed, F:

$$F = 2\ \mathrm{An} \int_0^1 C_b^*\, dz^*$$

where the absorption number, $\mathrm{An} = L/R \cdot P_w/\langle v_z \rangle$, L and R are the intestinal length and radius, P_w is the unbiased drug wall permeability, $\langle v_z \rangle$ is the axial fluid velocity, $C_b^* = C_b/C_o$ and is the dimensionless bulk or lumen drug concentration, C_b and C_o are the bulk and initial drug concentrations, respectively, and z^* is the fractional intestinal length and is equal to z/L. Three theoretical cases are considered: (I) $C_o \leq S$, $C_m \leq S$, (II) $C_o > S$, $C_m \leq S$, and (III) $C_o > S$, $C_m > S$, where S is the drug solubility and C_m is the outlet drug concentration. Solving the general steady-state mass balance result for fraction dose absorbed using the mixing tank (MT) and complete radial mixing (CRM) models results in the expressions for the fraction dose absorbed in humans. Two previously published empirical correlations for estimating fraction dose absorbed in humans are discussed and shown to follow as special cases of this theoretical approach. The MMBA is also applied to amoxicillin, a commonly prescribed orally absorbed β-lactam antibiotic for several doses. The parameters used in the correlation were determined from *in situ* or *in vitro* experiments along with a calculated system scaling parameter. The fraction dose absorbed calculated using the MMBA is compared to human amoxicillin pharmacokinetic results from the literature with initial doses approximated to be both above and below its solubility. The results of the MMBA correlation are discussed with respect to the nonpassive absorption mechanism and solubility limitation of amoxicillin. The MMBA is shown to be a fundamental, theoretically based model for estimating fraction dose absorbed in humans from *in situ* and *in vitro* parameters from which previously published empirical correlations follow as special cases.

KEY WORDS: extent of absorption; macroscopic mass balance analysis; mixing tank; complete radial mixing model; solubility; oral drug absorption; amoxicillin.

INTRODUCTION

Estimating the extent of oral drug absorption and drug absorption variation in humans can considerably aid in the selection of therapeutic candidates in the drug discovery process as well as assist the pharmaceutical scientist in identifying ways to optimize oral drug delivery in patients. Two fundamental reasons for developing predictive oral drug delivery models are (i) to make reasonable estimates of drug absorption without performing *in vivo* studies in humans and (ii) to gain a better understanding of the rate-limiting processes affecting drug absorption so that oral drug delivery strategies can be developed. The scope of this paper is to develop the general macroscopic mass balance approach with respect to absorption and solubility considerations and to demonstrate several correlations of *in vitro* and *in situ* parameters to *in vivo* results using the MMBA. In subsequent publications, the extension of the theory to include chemical and enzymatic reaction will be presented.

BACKGROUND

Intestinal Drug Absorption Models

There are a number of oral drug absorption models (1–7) that have utilized physicochemical, physical, and/or physio-

[1] College of Pharmacy, The University of Michigan, Ann Arbor, Michigan 48109-1065.
[2] Therapeutic Systems Research Laboratories, Inc., P.O. Box 7062, Ann Arbor, Michigan 48107.
[3] To whom correspondence should be addressed.

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logical factors affecting drug absorption from the gastrointestinal tract. The pH-partition hypothesis, the first such theory to stress the importance of physicochemical factors in the absorption process, was reported by Shore and coworkers (1) in 1957. Further advances in physicochemical oral absorption modeling focused primarily on correlations involving *in vitro* experiments such as dissolution studies and partition coefficient determinations. Ho *et al.* (8) suggested that the relationship between the apparent *n*-octanol/water partition coefficient and passive membrane permeability is complex and that a simple correlation usually can not be realized. While the correlation between partition coefficient and passive membrane permeability may exist for a homologous series of compounds (8), this relationship has been found to be inconsistent among a diverse group of drugs. Dressman *et al.* (2) demonstrated that for compounds where the permeability ratio (aqueous to membrane permeability) is proportional to the membrane–water partition coefficient, an absorption potential parameter can be calculated and correlated to fraction absorbed. The absorption potential is an empirical correlation that is satisfactory for drugs absorbed by a membrane partitioning mechanism. The absorption potential correlation is not useful, however, for nonpassively absorbed compounds such as the β-lactam antibiotics or the ACE inhibitors since the absorption of these compounds occurs, at least in part, by a carrier mechanism of unknown specificity (9). Moreover, the intestinal transport of small, hydrophilic drugs may occur by a paracellular transport pathway that is charge and molecular size dependent. A membrane partitioning-based correlation would not be expected to hold in this case either.

Using a physical approach Amidon *et al.* (3), Dressman and Fleisher (4), Goodacre and Murray (10), Ho *et al.* (8), and Johnson and Amidon (11) have discussed the three fundamental resistances to mass transfer encountered in the absorption process. The three physical barriers to drug absorption are the dissolution resistance, aqueous boundary layer resistance, and membrane resistance. The importance of obtaining physiologically and physically meaningful values of these resistances using *in vitro* methods is essential for making *in vivo* estimates. If meaningful estimates of these resistances cannot be obtained, then only simple correlations of the rank-order type are possible. While the aqueous boundary layer and dissolution resistances are dependent upon the fluid hydrodynamics of the particular system, the intrinsic wall permeability is independent of these effects. Therefore, the dimensionless wall permeability determined from *in situ* intestinal perfusion studies in rats may be used as an estimator of membrane transport in humans. The assumption that the membrane permeability in rat correlates with that in humans (8) is fundamental for such correlations. Evidence to support this hypothesis was presented recently by Amidon *et al.* (3), where they reported on a correlation between the mean intestinal wall permeability of drugs in the perfused rat jejunal segment and the extent of absorption in humans. They found that, unlike the partition coefficient correlations, the relationship between the extent of absorption and the mean wall permeability was independent of the drug's structural class or absorption mechanism. It was also concluded that while other parameters such as the partition coefficient or pK_a may serve as useful guides, the fundamental absorption parameter of interest is the wall permeability.

Clinical Pharmacology of Amoxicillin

Amoxicillin is a broad-spectrum, bactericidal antibiotic given orally for the treatment of various gram-positive and gram-negative infections. In typical clinical dosing regimens, 250 or 500 mg is given by mouth three (TID) or four (QID) times daily. A 250-mg dose usually results in peak plasma levels within 1 hr, with peak plasma levels of about 4 μg/ml (12). Assuming that 200 ml of water is taken with a 250- or 500-mg dose, the initial intestinal concentration of amoxicillin is estimated to be 1.25–2.50 mg/ml. Furthermore, the solubility of amoxicillin is 5.66 to 6.33 mg/ml at pH 5.98 to 6.5 (13), roughly the physiological pH range of the human small intestine. Therefore, the initial estimated intestinal concentration of amoxicillin is substantially below its solubility for standard therapy.

For certain infections, clinicians are providing an alternative to the standard 14-day amoxicillin therapy (14–17). Single high-dose antimicrobial therapy has been proposed as a viable therapy for the approximately 4 million women who annually seek treatment for acute urinary tract infections (AUTI) (15–17). The major advantage to single-dose therapy is that it is less expensive than either conventional therapy or prophylaxis for recurrent infections (18,19). Other advantages of single-dose therapy include enhanced compliance and a reduced incidence of side effects (14,16). A number of drugs such as amoxicillin, trimethoprim, tetracycline, nitrofurantoin, and cephalexin have been shown to have higher cure rates than placebo when large single doses were administered (16). Three grams of amoxicillin given orally as one dose has been demonstrated to be safe and effective (16) for the treatment of AUTI. In addition to AUTI, single-dose amoxicillin therapy is also recommended by the American Heart Association for the prophylaxis of infective endocarditis (14). They recommend that a single oral dose of 3 g amoxicillin be administered to patients at risk prior to dental, genitourinary, and gastrointestinal tract procedures. For a patient taking a single 3-g dose of amoxicillin with 200 ml of water, the initial intestinal concentration of amoxicillin is about 15 mg/ml—more than twice its *in vitro* solubility at physiological pH.

In addition to solubility concerns, amoxicillin absorption is further complicated by a nonpassive absorption mechanism observed in rats (20) and humans (12,21,22). Several pharmacokinetic studies have been reported in the literature supporting both dose-dependent absorption due to a nonpassive absorption mechanism and solubility-limited absorption (12,21,22) for amoxicillin. For example, Spyker *et al.* (12) reported that at doses as high as 1000 mg, slower but complete absorption was observed in humans. In another study, Sjovall *et al.* (21) observed that with amoxicillin doses of 1500 and 3000 mg, slower and delayed absorption was observed. However, at this dose level the extent of absorption also decreased dramatically from the usual 85–90% to about 45%.

The MMBA is a flow model approach that uses scaling and mass transport concepts for estimating the extent of absorption for passively and nonpassively absorbed drugs in

humans. The MMBA also provides a theoretical framework for estimating fraction dose absorbed[4] in humans using parameters determined from *in vitro* and *in situ* experimental methods. In this report the general theoretical approach is developed and two previously published empirical correlations are shown to follow as special cases (2,3) of the MMBA. Furthermore, the MMBA is applied to published clinical human data for a commonly prescribed β-lactam antibiotic, amoxicillin, for doses above and below its solubility.

THEORETICAL

Mass Balance Considerations

For the following theoretical analysis a steady-state mass balance approach is adopted for predicting fraction dose absorbed in humans. The model of the small intestine used for the simultaneous absorption and flow of drugs is taken to be a cylinder with surface area of $2\pi RL$ (Figure 1) where R is the radius and L is the length of the tube. The rate of mass entering the tube is the product of the inlet concentration, C_o, and the volumetric flow rate, Q. The rate of mass exiting the tube is the product of C_m, the outlet concentration, and the volumetric flow rate. Assuming that mass is lost from the tube by absorption and mass flow out of the tube, the mass absorbed per unit time is the difference between the rate of mass flow in and out of the tube. Therefore, the rate of mass absorbed from the intestine is

$$-\frac{dM}{dt} = Q(C_o - C_m) = \int\int_s J_w \, dA \qquad (1)$$

where J_w is the wall flux and A is the absorptive surface area. For the initial theoretical development a passive absorption mechanism is assumed, however, extending the analysis to include a carrier-mediated (nonlinear) absorption mechanism is accomplished by using a mean wall permeability (3).

The mass absorbed across the surface area of the intestine per unit time is

$$-\frac{dM}{dt} = AJ_w \qquad (2)$$

where the general expression used for wall flux is

$$J_w = P_e C_b \qquad (3)$$

The wall flux at steady state is the product of the effective drug permeability, P_e, and the bulk drug concentration, C_b. These two factors are considered in more detail in the next section.

Concentration and Permeability Considerations

As seen in Eq. (3), the components of the wall flux are the intestinal wall permeability and the bulk drug concentration. The effective permeability, P_e, is defined as a function of the aqueous resistance, R_a, and wall resistance, R_w, normally associated with membrane transport when $C_o < S$ (C_o is the initial drug concentration and S is the drug's solubility). However, when $C_o > S$ it can be generalized to include the particle boundary layer resistance, R_s (8). Since $R = 1/P$ (permeability), and if it is assumed that particles do not enter the aqueous boundary layer,[5] then the effective resistance, in terms of permeability, becomes

$$\frac{1}{P_e} = \frac{1}{P_w} + \frac{1}{P_{aq}} + \frac{1}{(SA_p/SA_m)P_s}$$

where SA_p and SA_m are the total particle and wall surface areas. This result, based on a total mass balance, makes explicit the dependence on the surface area of the particle. Hence the particle boundary layer resistance can be considered included in P_e when $C_o > S$.[6]

The other component of the wall flux, the bulk drug concentration C_b, is treated by examining the inlet and outlet volume element concentrations relative to the drug's solubility. The inlet and outlet concentrations are partitioned into three cases: *Case I*—the inlet and outlet drug concentrations are always below the solubility (or $C_o \leq S$, $C_m \leq S$); (ii) *Case II*—the drug concentration initially exceeds the solubility but is in solution before exiting the tube (or $C_o > S$, $C_m \leq S$); and (iii) *Case III*—the drug concentration is always above the solubility, therefore, particles exit the tube (or $C_o > S$, $C_m > S$). The bulk drug concentration profile in the tube is dependent on the flow model chosen. Since the two ideal flow models used in this analysis do not account for radial concentration variation there is no distinction made between P_e and P_w. However, the operative permeability used in the *correlations* is the experimentally determined intrinsic wall permeability, P_w, from the *in situ* single-pass perfusion experiment in rats (3).

The Development of the Absorption Number

Setting Eq. (2) equal to Eq. (3) and applying the assumptions of cylindrical geometry and constant permeability. Eq. (1) becomes

$$-\frac{dM}{dt} = Q(C_o - C_m) = 2\pi R \, P_e \int_0^L C_b \, dz \qquad (4)$$

Introducing the dimensionless variables, S^*, z^*, and C_b^*,

$$S^* = S/C_o$$
$$z^* = z/L$$
$$C_b^* = C_b/C_o$$

where S/C_o is the dimensionless solubility, z/L is a fractional length, and the dimensionless concentration is C_b/C_o.

Equation (4) simplifies to

$$F = 1 - \frac{C_m}{C_0} = \frac{2\pi RL}{Q} P_e \int_0^1 C_b^* \, dz^* \qquad (5)$$

[4] Fraction dose absorbed is defined in this report to be the fraction of drug lost from the lumen of the tube through the tube wall. It does not include other factors such as first-pass hepatic metabolism that may further reduce the drug's systemic availability.

[5] This is a frequently made implicit assumption.

[6] For simplicity in this report, it is assumed that dissolution is not rate limiting. Consequently, this analysis will predict the maximum extent of drug absorption when dosed above its solubility.

Substituting the volumetric flow rate, Q, which equals $\pi R^2 \langle v_z \rangle$ into Eq. (5) gives

$$F = 2 \frac{L}{R} \frac{P_e}{\langle v_z \rangle} \int_0^1 C_b^* \, dz^* \tag{6}$$

where L and R are the intestinal length and radius, and $\langle v_z \rangle$ is mean axial fluid velocity. It should be noted that this analysis is based on system average values. Stochastic variation of P_e and $\langle v_z \rangle$ can be included in the analysis and will be discussed in a subsequent report.

Equation (6) shows the four key factors affecting the extent of drug absorption: (i) a mass transfer (absorption) parameter, the effective drug permeability, P_e; (ii) a convection (bulk fluid flow) parameter, the mean axial fluid velocity ($\langle V_z \rangle$); (iii) geometric parameters, L and R; and (iv) the drug concentration profile that determines the value of the integral in Eq. (6). This suggests the definition of a new dimensionless absorption-convection parameter—the absorption number, An—which is defined as

$$\text{An} = \frac{L}{R} \frac{P_e}{\langle v_z \rangle} \tag{7}$$

Substitution of Eq. (7) into Eq. (6) results in the general relationship between the fraction dose absorbed and An:

$$F = 2 \text{ An} \int_0^1 C_b^* \, dz^* \tag{8}$$

Variations in An with length can be included. For example, considering small and large intestine tube segments gives

$$F = 2 \text{ An}_{SI} \int_0^L C_b^* \, dz^* + 2 \text{ An}_{LI} \int_L^1 C_b^* \, dz^*$$

where An_{SI} and An_{LI} are the absorption numbers for the small and large intestine, respectively. The initial analysis is restricted to small intestinal permeabilities and absorption.

The concentration profile in the tube, needed to evaluate the integral in Eq. (8), is dependent upon the flow model that is chosen for the analysis. In the next section two ideal flow models are considered.

Flow Model Considerations

The simplest of these models is the completely stirred or well-mixed tank (MT). The contents of the steady-state MT, as its name suggests, are well stirred and uniform throughout, with instantaneous dilution of the inlet stream occurring in the tank. Mixing tank models have been used to predict dissolution rate and nondissolution rate-controlled absorption (4).

Plug flow and complete radial mixing (CRM) models (23) on the other hand, are also commonly used to model the oral absorption process (3,5). The CRM model represents the other extreme of flow patterns, namely, where there is no axial mixing of fluid elements. The CRM is particularly useful for modeling spatially dependent processes. The mass balance on the CRM reactor is based on a differential volume element. Therefore, some of the differential volume elements in the Case II CRM reactor exist in Case I, while the remainder are in Case III. This results in an apparent intermediate case for the tube as a whole, although each differential volume element exists in either Case I or Case III. This is not possible with the MT reactor since mixing is instantaneous and complete. Although fundamental differences exist between the MT and the CRM models the analysis is consistent since the mass balance is always taken on the volume element rather than the tube (or reactor).

Since the mass balance volume elements of both models chosen for this analysis are well stirred, there is no aqueous resistance; thus P_e and P_w can be used interchangeably in the notation. However, the intrinsic wall permeability used for the MMBA correlation is hydrodynamically unbiased and is determined from the single-pass perfusion experiment. The single-pass perfusion experiment utilizes laminar flow conditions to calculate the intrinsic wall permeability.

CASE I: $C_o \leq S$, $C_m \leq S$

Mixing Tank Model

The fraction of the dose that leaves the tube is equal to the ratio of the outlet or bulk concentration to the inlet concentration. Therefore, the fraction dose absorbed is $1 - C_b/C_o$. In terms of the previously introduced dimensionless wall concentration, C_b^*, the fraction dose absorbed is defined as

$$F = 1 - C_b^* \tag{9}$$

Rearranging Eq. (9) in terms of C_b^* results in

$$C_b^* = 1 - F \tag{10}$$

Substituting Eq. (10) into Eq. (8):

$$F = 2 \text{ An} \int_0^1 (1 - F) \, dz^* \tag{11}$$

Integrating and simplifying Eq. (11) results in the relationship between the fraction dose absorbed and the absorption number for the mixing tank model in Case I:

$$F_I = \frac{2 \text{ An}}{(1 + 2\text{An})} \tag{12}$$

Complete Radial Mixing Model

For the CRM model the concentration profile of drug in the intestine is

$$C_b^* = e^{-2 \text{ An } z^*} \tag{13}$$

Recalling Eq. (8),

$$F = 2\text{An} \int_0^1 C_b^* \, dz^*$$

Substituting Eq. (13) into Eq. (8) it follows that

$$F = 2\text{An} \int_0^1 e^{-2 \text{ An } z^*} \, dz^* \tag{14}$$

Integrating Eq. (14) results in the following expression for F_I, the fraction dose absorbed for the CRM model, Case I:

$$F_I = 1 - e^{-2 \text{ An}} \tag{15}$$

CASE III: $C_o > S$, $C_m > S$

MT and CRM Flow Models

The solution for Case III is presented now since the solutions for Case I and Case III are utilized to calculate F_{II} for CRM. The drug concentration at the wall in the volume element of the CRM reactor is equal to the solubility of the drug. Since C_b^* is constant and equal to the solubility for each of the differential volume elements in the CRM reactor, it can be treated as a single volume element, in other words, it can be mathematically treated as a MT model. Substituting $C_b = S$ in for the concentration profile in Eq. (8), and performing the integration results in the fraction dose absorbed for Case III, F_{III}, for the MT and CRM flow models:

$$F_{III} = 2\ An\ S^* \tag{16}$$

CASE II: $C_o > S$, $C_m \leq S$

CRM Flow Model

The model used for the derivation of the fraction dose absorbed in Case II takes advantage of the spatially distributed characteristics of CRM and is illustrated in Fig. 1. The full derivation is found elsewhere (20). The amount of drug absorbed in Region 1 of the tube is defined as $F_I A_T$, where A_T is the total amount of drug. The amount entering Region 2 is then $(1 - F_I)A_T$. The amount of drug absorbed in Region 2 is $F_{II}(1 - F_I)A_T$. Therefore, the fraction dose absorbed for Case II, F_{II}, is

$$F_{II} = F_{III} + F_I(1 - F_{III}) \tag{17}$$

The fraction dose absorbed in Region 1 of the tube is

$$F_{II}^1 = 2\ An\ S^*\left(\frac{X_p}{L}\right) = 1 - S^* \tag{18}$$

whereas the extent of absorption in Region 2 is

$$F_{II}^2 = 1 - e^{\left(\frac{1}{S^*} - 2\ An\right)} \tag{19}$$

Therefore, the total fraction dose absorbed for Case II complete radial mixing is

$$F_{II} = 1 - S^*\ e^{\left(\frac{1}{S^*} - 1 - 2\ An\right)} \tag{20}$$

Mixing Tank Model

The solution for the Case II mixing tank model is the same as the Case I solution since, by definition of case II, the outlet drug concentration in the tube is less than the solubility.

$$F_{II} = \frac{2\ An}{(1 + 2An)}$$

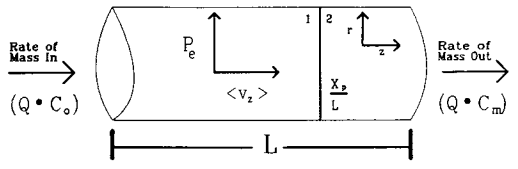

Fig. 1. Macroscopic mass balance on a tube. The rate of mass absorbed equals the difference between the rate of mass flow in and the rate of mass flow out of the tube. The absorption number (An) is an absorption–convection mass transfer number and is equal to the ratio of the radial mass transfer velocity (P_e) to the axial fluid velocity times a scaling factor.

METHODS

Application of the MMBA to Human Pharmacokinetic Data

The extent of absorption for amoxicillin is calculated using the CRM model and the corresponding equations in Table I. The *in vitro* solubility was used as reported from the literature and the *in situ* intestinal wall permeability was determined experimentally in rats. The volume given with the dose was taken as the intestinal volume.

Nonlinear Wall Permeability

For compounds that are absorbed by a carrier-mediated process, a concentration-dependent permeability requires the use of a mean permeability for these simulations (3). The mean permeability of a drug absorbed by a carrier-mediated process was previously defined as

$$\overline{P_w^*} = \frac{\int_{C_o}^{0} P_w^*\, dC}{\int_{C_o}^{0} dC}$$

where $C_o = D_o/V_L$, D_o is the dose administered, and V_L is the volume of the intestinal lumen. The final form of the mean permeability (3) is as follows.

Case I.

$$\overline{P_w^*} = P_m^* + P_c^* \frac{K_m}{C_o} \ln\left(1 + \frac{C_o}{K_m}\right) \tag{21}$$

Case III. By assumption $C_w \equiv S$ for Case III; therefore, the wall permeability at a given concentration is calculated in the usual way:

$$P_w^* = P_c^* / \left(1 + \frac{S}{K_m}\right) + P_m^* \tag{22}$$

Case II. For case II, the nonlinear wall permeability is a function of the Case I mean permeability [Eq. (21)], the Case III wall permeability [Eq. (22)], and the fractional length X_p/L. X_p/L is the fractional tube length where the Case III-to-Case I transition occurs. The algorithm for calculating the wall permeability for Case II was detailed previously (20) and is based on the following equation:

Table I. The Summary of Solutions for the Macroscopic Mass Balance Analysis for Predicting Fraction Dose Absorbed Using the Complete Radial Mixing and Mixing Tank Models for the Three Cases of Drug in Solution

Case	MT	CRM
I	$2\ An/(1 + 2An)$	$1 - e^{-2\ An}$
II	$2\ An/(1 + 2An)$	$1 - S^*\ e^{(1/S^* - 1 - 2\ An)}$
III	$2\ An\ S^*$	$2\ An\ S^*$

$$P_w^*(\text{II}) = X_p/L(P_w^*)^{\text{I}} + (1 - X_p/L)(P_w^*)^{\text{III}}$$

where $(P_w^*)^{\text{I}}$ is the Case I (mean) permeability, $(P_w^*)^{\text{III}}$ is the wall permeability at S, and X_p/L is the fractional length at which all of the drug is considered to be in solution.

The amoxicillin permeability data were determined from the rat intestinal perfusion experiment *in situ* (20). For amoxicillin the mean (SEM) absorption parameters calculated from derivative free nonlinear regression analysis are

K_m = 9.1 mM (4.0) (approximately 3.33 mg/ml)
P_c^* = 0.65 (0.07)
P_m^* = ND 0 (not different from 0)

where K_m is the absorption Michaelis constant, P_c^* is the carrier permeability, and P_m^* is the passive membrane permeability determined from the *in situ* single-pass perfusion experiment.

Amoxicillin Pharmacokinetic Data

Five sets of human data are used for these correlations (12,20,21,24,25). In a study by Spyker *et al.* (12), a group of eight subjects was given amoxicillin oral and intravenous doses of 250, 500, and 1000 mg. The data were analyzed using a two-compartment open model. The calculated first-order absorption rate constants for the three doses decreased from 1.23 hr^{-1} at 250 mg to 0.66 hr^{-1} at 1000 mg. Peak serum levels did not increase proportionally with dose, indicating dose-dependent absorption.[7] The extent of absorption was approximately the same for all three doses suggesting slower but complete absorption.

In a study by Sjovall *et al.* (21), amoxicillin was given in single doses of 375, 750, 1500, and 3000 mg in a randomized study to 12 fasting subjects. As with the previous study, C_{max} did not increase proportionally with dose and the t_{max} doubled from the lowest dose to the 3-g dose, indicating slower absorption with increasing dose.

In a study by Welling *et al.* (22), the influence of fluid volume on the relative bioavailability of amoxicillin was studied in six healthy human subjects. A 500-mg dose was given with either 250 or 25 ml of water. When 25 ml was given a marked decrease in bioavailability was observed. Furthermore, a similar pattern to the other two studies was observed, namely, an increase in t_{max} and less than proportional increase in C_{max}.

Finally, in a study by Arancibia *et al.* (25), the relative bioavailability of three doses of amoxicillin (500, 750, and 1000 mg) was determined in 12 healthy volunteers. Each dose was given with 200 ml of water. The relative bioavailability was calculated by comparing the amount of antibiotic excreted unchanged in the urine at 24 hr to the 250-mg dose. The relative bioavailability was 93.8, 98.2, and 76.8% for the 500-, 750-, and 1000-mg doses, respectively.

RESULTS AND DISCUSSION

The macroscopic mass balance analysis applies mass transfer concepts with a flow model approach to estimate the extent of drug absorption in humans. The two ideal flow models used in the analysis are the completely mixed tank and the complete radial mixing model. Furthermore, a dimensionless mass transfer coefficient—the absorption number, An—has been introduced. An is the ratio of the fundamental mass transfer processes affecting drug removal from the intestine: absorption and convection,

$$\text{An} = \frac{L}{R}\frac{P_e}{\langle v_z \rangle}$$

Substituting $P_e = P_w(1 - P_e/P_a)$ into the above equation results in

$$\text{An} = \frac{L}{R}\frac{1}{\langle v_z \rangle} P_w\left(1 - \frac{P_e}{P_a}\right)$$

For the MT and CRM models P_{aq} is infinite ($R_{aq} = 0$), therefore the relationship between An and P_w becomes

$$\text{An} = \frac{L}{R}\frac{P_w}{\langle v_z \rangle}$$

where P_w is the wall permeability of the drug, $\langle v_z \rangle$ is the mean axial fluid velocity, and L and R are the intestinal length and radius, respectively. As seen from the above equation there is no theoretical difference between P_e and P_w for either the MT or the CRM model. Dimensionless numbers typically include scaling or residence time distribution (RTD) factors and a mass transfer coefficient. Scaling factors include system size or geometry (L and R) and fluid property variables such as the average fluid velocity ($\langle v_z \rangle$). The mass transfer coefficient incorporated into An is the wall permeability of the drug. As seen in Fig. 1, the two mass transfer velocity terms of the absorption number are the radial mass transfer velocity (P_w) and the mean axial fluid velocity (the convective component). Assuming that the drug in solution moves axially at the same velocity as the fluid, then $\langle v_z \rangle$ represents the velocity of mass flow out of the tube, whereas the wall permeability is the radial mass transfer velocity through the intestinal membrane. P_w is the experimentally calculated and hydrodynamically unbiased intestinal wall permeability of the drug calculated from the single-pass perfusion experiment (3) and is fundamental to the MMBA derived herein. Although the wall and effective permeabilities are indistinguishable for the two ideal flow models used in this analysis, it is has been previously shown that the experimentally determined (unbiased) wall permeability should be used (3) for the MMBA correlations.

The MMBA provides a general theoretical framework for estimating the extent of drug absorption in humans. As shown below, two previously published correlations (2,3) follow from this general theoretical approach.

Case I Correlation: Fraction Dose Absorbed and P_w^*

In an earlier report (3), the fraction dose absorbed in humans was shown to correlate with the dimensionless intestinal wall permeability measured in a perfused rat intestinal segment. Using estimated intestinal parameters in humans, they demonstrated a good correlation between the fitted and the estimated Graetz number as well as between F and P_w^*.

[7] The intravenous pharmacokinetics in this study were dose linear.

The previously published correlation (3) between the rat permeability (P_w^*) and the human extent of absorption data can be derived from the MMBA as follows. When the absorption number is separated into its scaling or RTD and mass transport components, converted to dimensionless permeabilities, and the parameters are grouped, the expression for An becomes a function of the two parameters, P_w^* and Gz, found to correlate with F in the previous publication,

$$\text{An} = \frac{L}{R}\frac{P_w}{\langle v_z\rangle} = \frac{D_{aq}L}{R^2\langle v_z\rangle}P_w^* = P_w^*\,Gz$$

where the Graetz number, Gz, differs from the previous report (3) by a factor of two since the average velocity in laminar flow systems used in the previous correlation is equal to one-half maximal velocity. This is not the case for the CRM and MT models.

The MMBA correlation requires the use of a flow system scaling parameter. The flow system scaling or RTD parameters are dependent upon the axial fluid velocity and geometric dimensions of the flow system, therefore, it is necessary to calculate scaling parameters for each flow model. The scaling parameter of the rat permeability data to human fraction absorbed data was obtained for each flow model by fitting the data to Eqs. (12) and (15) using the above form of An. The scaling parameters were estimated using nonlinear regression (SYSTAT Nonlin-Simplex, Evanston, IL). For the mixing tank and complete radial mixing models, the mean (standard error) was 2.28 (0.39) and 1.27 (0.14), respectively. Using the fitted scaling parameters, absorption numbers for the MT and CRM models were calculated. The theoretical curves from Eqs. (12) and (15) for Case I using the MT and CRM models are shown in Fig. 2 along with the calculated An data. As expected, the case I correlation between the extent of absorption and An for both nonpassively and passively absorbed drugs works as well as the previously published F and P_w^* correlation. However, the significance of the MMBA over the previous F versus P_w^* correlation is that the MMBA truly separates the RTD or scaling parameter from the drug absorption properties and that it extends the earlier correlations to include solubility limited absorption. In the previous correlation, Gz was considered a scaling parameter, however, Gz includes the drug's aqueous diffusivity (D_{aq}). Gz is most appropriately used in laminar flow systems where the radial diffusion time and axial convection time are important. For the two ideal flow models used in the MMBA, D_{aq} plays an unimportant role since radial diffusion occurs instantaneously. It is important to emphasize that these initial correlations use average system values. The stochastic variation in flow rate and permeability can be included in the analysis and is the subject of a preliminary report (26).

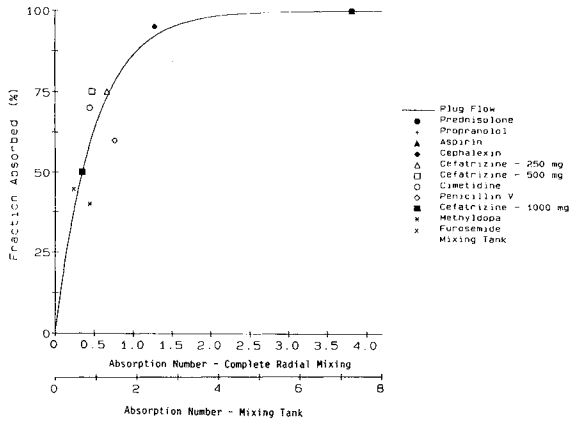

Fig. 2. Plot of the extent of absorption versus the absorption number (An)—complete radial mixing or absorption number—mixing tank for various passively and nonpassively absorbed compounds. The solid line represents the simulated curve using the MMBA and the complete radial mixing model, whereas the dotted line represents the data simulated using the MMBA and the mixing tank model.

Case III Correlation: Absorption Potential Derivation

While the fraction dose absorbed for drugs in Case I is dependent upon An, the fraction dose absorbed for Cases II and III requires an additional parameter—the dimensionless solubility (S^*)—in order to estimate F. S^* is used rather than S since the solubility of each compound at a given absorption number usually differs, making comparisons of drugs with equal An difficult. Normalizing the solubility by the initial dose and volume given allows all drugs with equal absorption numbers to be compared on a dose basis.

The absorption potential approach (2) is the second special case of the MMBA. The AP was originally proposed as a correlating parameter for estimating drug absorption in humans. The AP is an *empirical* approach based primarily on the physicochemical properties of the drug where the AP is equal to[8]

$$\text{AP} = PC\,f_u\,S^*$$

where PC is the partition coefficient, f_u is the fraction nonionized, and S^* is the dimensionless solubility. Taking a simple lipid partitioning membrane model for P_e (i.e., assuming only nonionized drug penetrates the membrane),

$$P_e = D_m PC/\delta_m f_u$$

it can be shown that

$$\text{AP} = \alpha\,\text{An}\,S^*$$

where $\alpha = 2R\delta_m/(D_{aq}D_m)$, D_{aq} and D_m are the drug's aqueous and membrane diffusion coefficient, respectively, R is the intestinal radius, and δ_m is the membrane thickness. Consequently, the absorption potential parameter follows directly from Case III of the MMBA and a membrane partitioning model assumption.

Since a membrane partitioning model was assumed for the original AP, the relationship is applicable only to passively absorbed drugs. The advantage of using An over AP is that both passively and nonpassively absorbed drugs can be included in the correlation. Nonpassively absorbed drugs (e.g., β-lactam antibiotics, ACE inhibitors, and α-methyl DOPA) are included in the MMBA correlation by using the mean wall permeability (3) to account for the concentration dependence in the absorption number.

[8] The absorption potential in the previous publication was taken as $\log(PC\ f_u\ S^*)$. It is more convenient to define it as above.

It is also noted that, in an extended analysis of the absorption potential, Macheras and Symillides (27) concluded that overestimating AP is avoided if the value of one is assigned to the solubility–dose term in cases where $S_o > X_o/V_L$. The MMBA provides the theoretical basis for this conclusion. In the case when the initial drug concentration is below the solubility the extent of drug absorption correlates with An, whereas for initial drug concentrations that exceed the solubility the correlating variable becomes AnS^*.

APPLICATION OF THE MMBA TO AMOXICILLIN DATA IN HUMANS

Amoxicillin is an ideal model compound for validating the MMBA for Cases I, II, and III since a wide range of doses has been studied in humans and the β-lactam antibiotics are relatively nontoxic at even large doses (28). Furthermore, pharmacokinetic evidence appears to support two mechanisms for the delay and/or decrease in amoxicillin absorption with increasing dose: (a) a dose-dependent absorption mechanism and (b) a solubility/dissolution effect.

Nonpassive Absorption

Nonpassive absorption of amoxicillin has been observed in both humans (12,21,22) and rats (20). In the Spyker study (12) a significant decrease in the first-order absorption rate constant from 1.23 to 0.66 hr^{-1} was reported. The initial concentration (dose per volume taken) in that study was always below the solubility, suggesting that the effects are due to nonlinear absorption rather than a solubility limitation. Other pharmacokinetic parameters also exhibited dose-dependent absorption behavior. For example, the value of t_{max} increased while C_{max} increased less than proportionally due to slower absorption. The reported dose-*independent* extent of amoxicillin absorption (based on the percentage of the dose recovered in the urine and the AUC) and the resultant 50% decrease in the absorption rate constant suggest that, although absorption slowed with increasing dose, there was sufficient intestinal length to assure nearly complete absorption. The observed decrease in k_a can be explained based on the concentration dependence of P_w. It can be shown that $k_a = SA/V\,P_w$, where k_a is the mean absorption rate constant, SA is the intestinal surface area, V is the intestinal volume, and P_w is the mean intestinal drug permeability. Since P_w^* decreases with increasing drug concentration for nonpassively absorbed drugs, it can be demonstrated that k_a also decreases with increasing dose.

Solubility-Limited Absorption

In the Sjovall *et al.* study (21) the results were similar to the results of the Spyker *et al.* study for doses below the solubility. At the 1500- and 3000-mg doses, however, the AUC and UR% (% of dose recovered in the urine) declined significantly, suggesting that absorption was slowed significantly so that the intestinal residence time was less than the time required for complete absorption to occur. Therefore it is hypothesized that unabsorbed amoxicillin passed into the colon, where its absorption is substantially lower, resulting in a significant decrease in the extent of absorption. The intestinal concentrations at these doses exceeded the K_m by a factor of 2 to 5, suggesting a nonlinear absorption effect; however, the initial concentrations for both the 1500-mg and the 3000-mg doses were also significantly above the solubility (5.66–6.33 mg/ml) of amoxicillin (13).

The study by Welling *et al.* (22), demonstrates two extreme dosing cases. In the first case the initial amoxicillin concentration (500 mg/25 ml or 20 mg/ml) is almost four times greater than the solubility, whereas the other concentration studied is well below the solubility at 500 mg/250 ml or 2 mg/ml. The observed UR% decreased from 85% at the lower initial concentration to 49% at the higher initial concentration, suggesting that incomplete absorption had occurred.

The data in the five studies were combined for the purpose of performing the correlation and are shown in Table II. The maximal fraction dose absorbed was estimated from an i.v. and oral pharmacokinetic study (24). This value was determined to be approximately 80%; therefore, this was taken as the upper limit for F. The fraction dose absorbed data were combined into Table II and normalized to 80%. This was necessary to compare the dose and reported F between the literature studies used for the MMBA correlation. The reported standard deviations for the human data were also normalized for the correlation based on the coefficient of variation. Simulations were performed using the following mean system parameters: $P_c^* = 0.65$, $K_m = 9.1$ mM (from single-pass perfusion experiments in rats), intestinal volume = 200 ml (normalized volume given with dose), scaling parameter = 1.27, and mean amoxicillin solubility = 6.0 mg/ml. Once again, the volume given was normalized to 200 ml to facilitate comparisons between the studies. The mean solubility was the average of the reported solubility at pH 5.98 and 6.5 (13). The normalized human data and simulated curve (curve B) for amoxicillin are plotted in Fig. 3 for

Table II. Summary of Amoxicillin Pharmacokinetics and Calculations

Dose (mg/ml)	F(%, predicted)[a]	F(%, observed)	Ref. No.
1.25	75.3	76.6	12
1.25	75.3	82.5	25
1.88	73.0	76.7	21
2.00	72.5	76.5	22
2.50	70.8	77.4	25
2.50	70.8	77.4	24
K_m = 3.33 mg/ml			
3.75	66.8	81.3	25
3.75	66.8	64.9	21
5.00	63.5	73.8	25
5.00	63.5	63.4	12
Solubility = 5.66–6.33 mg/ml			
7.50	53.3	53.0	21
15.00	20.5	41.0	21
20.00	13.2	35.6	22

[a] The estimated fraction dose absorbed from the macroscopic mass balance analysis.

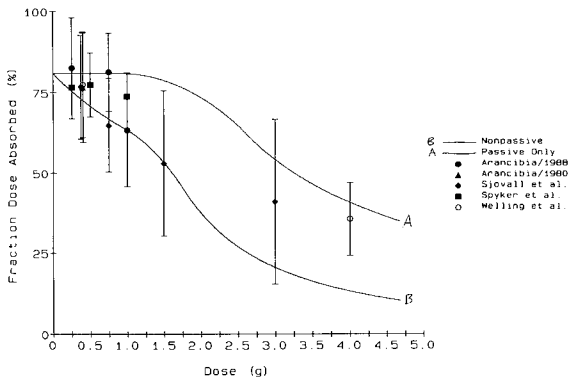

Fig. 3. Plot of fraction dose absorbed for amoxicillin versus the dose given. Curve A represents the simulated data using a passive absorption mechanism ($P_w^* = 0.65$), whereas curve B represents the simulated curve generated using a nonpassive absorption mechanism ($P_c^* = 0.65$). Both curves were simulated using the equations for CRM in Table I. The data points represent the normalized pharmacokinetic data from the five amoxicillin studies (see Table II).

the complete radial mixing flow model. As seen in Fig. 3 the simulation is in good agreement with the human data, even though dissolution rate was assumed to be non-rate limiting. In the calculations for Fig. 3 it is assumed that the volume given with the dose approximates the intestinal volume.

The effect of using a passive rather than a nonpassive absorption mechanism on the extent of amoxicillin absorption is shown in Fig. 3, curve A. For curve A, a passive permeability (P_m^*) equal to 0.65 was used, while curve B represents the nonpassive absorption simulation using a carrier permeability ($P_c^* = 0.65$). Due to the scatter in the human data, a case could be made for the use of either absorption mechanism, however, the use of the nonpassive absorption mechanism is justified from the evidence in the literature such as the Sjovall or Spyker studies. The use of the passive permeability represents the upper limit on the extent of absorption and since the actual intestinal concentration of amoxicillin will vary most observed data should fall between the two curves.

The application of the MMBA to amoxicillin demonstrates that the two key parameters controlling drug absorption are An and S^*. For the amoxicillin correlations in this report, mean experimental parameter values have been used. Variation in either the dose-to-K_m ratio, the dose-to-solubility ratio, or An as reflected in the value of parameters such as $\langle v_z \rangle$, P_w^*, or the intestinal volume will be considered in a future publication.

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