Calculation of the Aqueous Diffusion Layer Resistance for Absorption in a Tube: Application to Intestinal Membrane Permeability Determination

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Received June 14, 1990; accepted October 3, 1990

The single-pass intestinal perfusion technique has been used extensively to estimate the wall permeability in rats. The unbiased membrane parameters can be obtained only when the aqueous resistance is properly accounted for. This aqueous resistance was calculated numerically from a convective diffusive mass transfer model, including both passive and carrier-mediated transport at the intestinal wall. The aqueous diffusion layer resistance was shown to be best described by a function of the form,

\[ \frac{1}{P_{\text{aq}}^{-1}} = AG_{c}^{1/3} + BG_{c}^{C} \left[ P_{\text{m}}^{2} \left( \frac{K_{m}}{C_{o}} \right)^{D} + P_{n}^{n} \right]^{E} \]

where \( G_{c} \), \( P_{\text{m}} \), \( P_{n} \), \( K_{m} \), and \( C_{o} \) are, respectively, Graetz number, passive permeability, carrier-mediated permeability, Michaelis constant, and the drug concentration entering the tube. Asterisked are dimensionless quantities obtained by multiplying the permeability constants with \( R/D \), where \( R \) and \( D \) being radius and drug diffusivity, respectively. A, B, C, D and E were obtained by a least-squares nonlinear regression method, giving values of 1.05, 1.74, 1.27, 0.0659, and 0.377, respectively, over the range of 0.001 \( \leq G_{c} \leq 0.5 \), 0.01 \( \leq P_{\text{m}} \leq 10 \), 0.01 \( \leq P_{n} \leq 10 \), and 0.01 \( \leq K_{m}/C_{o} \leq 100 \). This aqueous resistance was found to converge to those calculated from Levich’s boundary layer solution in low Graetz range, indicating the correct theoretical limit. Using an iteration method, the equation was shown to be useful in extracting the intrinsic membrane permeability from the experimental data.

KEY WORDS: aqueous resistance; laminar tube flow; diffusion; intestinal absorption; carrier-mediated transport; permeability; numerical method.

INTRODUCTION

The intestinal permeability of a compound is commonly estimated by a single-pass perfusion technique. Assuming that there is no radial convection due to water transport, the overall diffusional resistance to absorption can be visualized as a composite of two resistances in series, i.e.,

\[ \frac{1}{P_{\text{eff}}} = \frac{1}{P_{\text{aq}}} + \frac{1}{P_{w}} \]  \( (1) \)

where \( P_{\text{eff}}, P_{\text{aq}}, \) and \( P_{w} \) denote effective, aqueous, and wall permeabilities, respectively. The effect of the aqueous resistance layer, also known as the unstirred or stagnant layer, has been clearly delineated both experimentally (1,2) and theoretically (3-6). \( P_{\text{eff}} \) can be calculated from experimental data according to a complete radial mixing model,

\[ P_{\text{eff}} = -\frac{Q}{2\pi RL} \ln \frac{C_{m}}{C_{o}} \]  \( (2) \)

where \( C_{m}/C_{o} \) is the measured fractional exit concentration, and \( Q, R, L \) are flow rate, radius, and length, respectively (7). By separating out the aqueous contribution, the true wall permeability can be obtained. In the most general case, \( P_{w} \) is expressed by passive and carrier-mediated mechanisms in parallel, i.e.,

\[ P_{w} = \frac{J_{\text{max}}}{K_{m} + C_{w}} + P_{m} \]  \( (3) \)

where \( J_{\text{max}}, K_{m}, \) and \( P_{m} \) are the maximum flux, Michaelis constant, and passive permeability, respectively. These quantities can be obtained by a nonlinear regression according to a variant form of Eq. (3) (9). If one ignores the aqueous layer, the membrane parameters obtained are biased, where \( K_{m} \) is always overestimated and \( P_{m} \) and \( J_{\text{max}} \) underestimated. Therefore, it is necessary to either eliminate or account for the effect of this aqueous resistance in order to arrive at the true membrane parameters.

Different approaches have been used to either minimize...
or account for the effect of this layer, such as maximizing the perfusion flow rate or simultaneous perfusion of fluid and air to introduce turbulence and reduce the concentration gradient in the lumen (1). Although these techniques have been shown to be effective in reducing the influence of the aqueous layer, the extent of this reduction is still unknown. Alternatively, one can account for the aqueous layer resistance theoretically through proper modeling of the hydrodynamics in the lumen. The advantage of the theoretical approach is that it allows unambiguous quantitation of the aqueous resistance, defined as $1/P_{aq}$, without resorting to an unknown “unstirred” layer thickness to fit the data.

Methods based on mass transport modeling to account for the aqueous resistance were previously developed (8,9). Based on the solution to a convective mass transfer problem with axial laminar flow in a straight tube, Elliott et al. (8) developed an approximate method for calculating the membrane permeability of passively absorbed compounds. The method is restricted to the passive diffusion at the intestinal wall because the solution scheme cannot accommodate non-linear boundary conditions, such as one involved in Michaelis Menton absorption. Subsequently, Johnson and Amidon used the boundary layer approach similar to the one used by Levich (12) to solve a laminar tube flow problem including both passive and carrier-mediated components in the boundary condition (9). Upon linearizing the axial parabolic velocity profile, a solution was obtained analytically on seminfinite coordinates. The aqueous resistance derived from the solution is expressed by

$$P_{aq}^{-1}(x) = 1.47 \frac{R}{D} G_z^{1/3} \left( \frac{x}{L} \right)^{1/3}$$  \hspace{1cm} (4)

where $R$, $L$, $D$, and $x$ are radius, length, aqueous diffusivity of the drug molecules, and axial coordinate, respectively; the Graetz number, $G_z$, is defined by

$$G_z = \frac{\pi DL}{2Q}$$  \hspace{1cm} (5)

where $Q$ is the volume flow rate in the tube. To improve the accuracy and extend the applicability of this method, the solution was adjusted by adding a parameter, $A$, to the aqueous resistance and matched to Elliott’s solution in the first-order case. This results in the modified boundary layer solution (MBLS), and the aqueous resistance obtained is expressed as

$$P_{aq}^{-1} = A \frac{R}{D} G_z^{1/3}$$  \hspace{1cm} (6)

with

$$A = \begin{cases} 10.0G_z + 1.01, & 0.004 \leq G_z \leq 0.01 \vspace{0.5cm} \\ 4.5G_z + 1.065, & 0.01 \leq G_z \leq 0.03 \vspace{0.5cm} \\ 2.5G_z + 1.125, & 0.03 \leq G_z \end{cases}$$

While the aqueous resistance estimated by this method has been used extensively in calculating member permeability, several issues have not yet been resolved. First, in the MBLS scheme, the aqueous resistance is found to be independent of the membrane parameters, while Elliott’s solution indicates that there is such a dependence. The significance of this dependence needs to be established. Second, the aqueous resistance of Eq. (6) has not been rigorously defined. Third, the accuracy of the MBLS has not been verified. To address these issues, a convective diffusive tube flow model was set up to analyze the concentration distribution within the tube interior. From these results, the aqueous resistance was quantitated and analyzed.

**THEORY**

The experimentally measured $P_{eff}$ is an averaged quantity over the entire length of the tube as evidenced in Eq. (2). Consequently, it follows that the $P_{aq}$ will necessarily be an averaged value as well. The purpose of the following derivation is to define this averaged quantity unambiguously. At steady state, the flux $J(z)$ through the intestinal membrane and aqueous boundary layer are equal, i.e.,

$$J(z) = P_{aq}(z)[C_c(z) - C_w(z)] = P_w(z)C_w(z)$$  \hspace{1cm} (7)

where sink conditions are assumed on the serosal side of the intestinal membrane. Rigorously, $J$, $P_{aq}$, and $P_w$ are $z$ dependent. $C_c$ and $C_w$ are the concentrations at the center axis and tube wall, respectively. Since a spatially averaged quantity is sought, a mean $P_{aq}$ can be defined by equating the integral membrane flux to the flux through a reference mean concentration gradient in the aqueous phase, i.e.,

$$\int_A P_wC_w dA = AP_{aq}(C_c - C_w)$$  \hspace{1cm} (8)

where the integral is evaluated over the intestinal membrane surface $A$. More generally, $P_w$ is considered to be a combination of passive diffusion and carrier-mediated mechanisms in parallel as indicated in Eq. (3). Substituting Eq. (3) into Eq. (8) and setting $A = 2\pi RL$, the following expression for $P_{aq}$ is obtained:

$$\frac{1}{P_{aq}} = \frac{\int_0^L \left( \frac{J_{max}C_w}{K_m + C_w} + P_wC_w \right) dz}{[L(C_c - C_w)]}$$  \hspace{1cm} (9)

The quantities $C_c$ and $C_w$, yet to be defined, are the reference concentrations at the center axis and intestinal wall, respectively. They are simply taken to be the averages of their respective axially dependent quantities, i.e.,

$$\overline{C}_c = \frac{\int_0^L C_w dz}{L}$$  \hspace{1cm} (10)

$$\overline{C}_w = \frac{\int_0^L C_c dz}{L}$$  \hspace{1cm} (11)

It is apparent that, in order to evaluate $P_{aq}$, one needs to know the concentrations at the wall and center axis. These quantities can be obtained by solving a mass transfer model with proper hydrodynamics incorporated.

The equation of continuity at steady state with axial convection and radial diffusion in cylindrical coordinates can be written as