Analysis of Intestinal Perfusion Data for Highly Permeable Drugs Using a Numerical Aqueous Resistance—Nonlinear Regression Method

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Purpose. To develop, validate and apply a method for analyzing the intestinal perfusion data of highly permeable compounds using the Numerical Aqueous Resistance (NAR) theory and nonlinear regression (NAR-NLR) and to compare the results with the well-established Modified Boundary Layer (MBL) Analysis.

Methods. The NAR-NLR method was validated and the results were compared to the MBL analysis results using previously reported cephradine jejunal perfusion data. Using the Single Pass Intestinal Perfusion (SPIP) method, the concentration dependence of intestinal permeability was investigated for formycin B, proline, and thymidine, three compounds reported to be absorbed by carrier-mediated transport processes. The MBL and NAR-NLR analyses were then applied to the three sets of SPIP data.

Results. The results demonstrate that the intrinsic MBL transport parameters were highly variable and, in one case, the analyses failed to give a statistically significant Michaelis constant. The MBL mean dimensionless wall permeabilities (P^*_w) were greater than the NAR-NLR P^*_w and were also highly variable. In all cases, the NAR-NLR variability was significantly lower than the MBL variability. The extreme variability in the MBL-calculated P^*_w is due to the sensitivity of P^*_w when the fraction of unabsorbed drug (C_m/C_o) is low or, alternatively, when P^*_w approached the aqueous permeability, P^*_{aq}.

Conclusions. The NAR-NLR method facilitates the analysis of intestinal perfusion data for highly permeable compounds such as those absorbed by carrier-mediated processes at concentrations below their K_m. The method also allows for the use of a wider range of flow conditions than the MBL analysis resulting in more reliable and less variable estimates of intestinal transport parameters as well as intestinal wall permeabilities.

KEY WORDS: numerical aqueous resistance; cephradine; formycin B; intestinal perfusion; intestinal permeability; modified boundary layer analysis, proline; thymidine.

INTRODUCTION

The single pass intestinal perfusion (SPIP) experiment is commonly used for characterizing the oral absorption mechanisms and intestinal transport properties of drugs and nutrients (1, 2). Assuming that the two resistances (resistance = 1/permeability) to drug absorption in a tube, the membrane and aqueous resistances, are in series then the effective resistance, in terms of permeability, is defined as,

$$\frac{1}{P_e} = \frac{1}{P_{aq}} + \frac{1}{P_w} \tag{1}$$

where P_e, P_{aq}, and P_w are the effective, aqueous, and wall intestinal permeabilities, respectively.

Experimentally, the effective permeability is calculated from a complete radial mixing model (3),

$$P_e = -\frac{Q}{2\pi RL} \ln \frac{C_m}{C_o} \tag{2}$$

where Q is the volumetric flow rate, R is the radius, L is the length, C_m is the outlet drug concentration and C_o is the inlet drug concentration. Permeabilities are made dimensionless using $P^* = P\,R/D_{aq}$ where D_{aq} is the estimate of the compound's aqueous diffusion coefficient.

Calculation of the P^*_w from P^*_e, requires an estimate of P^*_{aq}. Using a convective-diffusive tube flow model, Kou *et al.* (4) analyzed and quantitated the aqueous resistance resulting in the following empirical equation,

$$\overline{P^{*-1}_{aq}} = AGz^{1/3} + BGz^C\left[P^*_c\left(\frac{K_m}{C_o}\right)^D + P^*_m\right]^E \tag{3}$$

where the Graetz number, Gz, is

$$Gz = \frac{\pi D_{aq} L}{2Q} \tag{4}$$

and,

$$A = 1.05$$

$$B = 1.74$$

$$C = 1.27$$

$$D = 0.0659$$

$$E = 0.377$$

and K_m is the Michaelis constant, P^*_c is the carrier permeability and P^*_m is the passive membrane permeability.

Using an iterative method, P^*_w was then determined. The intestinal transport parameters were obtained by regressing P^*_w versus C_w using the following model for intestinal wall permeability:

$$P^*_w = \frac{P^*_c}{1 + \frac{C_w}{K_m}} + P^*_m \tag{5}$$

The iterative method can be used for drugs that are absorbed by both carrier-mediated and passive mechanisms; however, the method is difficult to use for carrier-mediated transport since two or three parameters (P^*_c, K_m, and, if appropriate, P^*_m) are involved in the iterative calculation. In this report, a straightforward method for determining intestinal wall permeability using the NAR solution of Kou *et al.* (4) and nonlinear regression analysis is developed and validated using cephradine data. The method is then successfully applied to formycin B, proline and thymidine. The NAR-NLR transport

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parameters and P^*_w are compared and contrasted to the results of the MBL analysis.

METHODS

Single Pass Intestinal Perfusion Data

The SPIP method and data for cephradine were previously reported in the jejunum of rats (2). Jejunual results were obtained for formycin B, proline and thymidine using established perfusion procedures (1, 2). Experimental parameters for formycin B were: L = 6.3–17.0 cm, Q = 0.191 ml/min, perfusate concentrations studied: 0.01mM, 0.03mM, 0.1mM, 0.3mM, 1mM, and 3mM. Experimental parameters for proline were: L = 5.5–10.3 cm, Q = 0.191 ml/min, perfusate concentrations studied: 0.01mM, 0.1mM, 1mM, 10mM, 100mM, and 200mM. Experimental parameters for thymidine were: L = 6.4–12.8 cm, Q = 0.191 ml/min, perfusate concentrations studied: 0.001mM, 0.01mM, 0.1mM, 1mM, 10mM, and 25mM. The composition of perfusion buffers was previously reported (5). All perfusion buffers were iso-osmotic (290mOsm/kg) and pH 6.5.

Data Analysis

Modified Boundary Layer Analysis

The MBL analysis is detailed elsewhere(6). Briefly, the effective permeability is calculated from the experimental data using Equation 2. P^*_{aq} is then estimated from the following equation,

$$P^{*-1}_{aq} = AGz^{(1/3)} \tag{6}$$

where,

$$A = 10.0\ Gz + 1.01, \quad 0.004 \le Gz \le 0.01$$

$$A = 4.5\ Gz + 1.065, \quad 0.01 \le Gz \le 0.03$$

$$A = 2.5\ Gz + 1.125, \quad 0.03 \le Gz$$

P^*_w is calculated from Equation 1 using the values of P^*_{aq} and P^*_e. The intrinsic membrane transport parameters are then found by regressing P^*_w versus C_w using Equation 5. The concentration of drug at the wall of the tube was previously defined as $C_w = C_o\ (1 - P^*_e / P^*_{aq})$.

Numerical Aqueous Resistance—Nonlinear Regression (NAR-NLR) Analysis

In order to perform the NAR-NLR analysis of perfusion data, C_o, P^*_e, and Gz are required. The data that is obtained from the SPIP experiment includes: C_o, C_m, L, and Q. P^*_e is calculated from $P^*_e = (1\text{-}(C_m/C_o)')/4Gz$. $(C_m/C_o)'$ is the ratio of outlet to inlet drug concentration corrected for net fluid flux. The Graetz number is calculated using Equation 4.

Determining the Intrinsic Absorption Parameters and P^*_w

Rearranging Equation 1 results in the following expression for dimensionless effective permeability,

$$P^*_e = \frac{P^*_{aq} P^*_w}{P^*_{aq} + P^*_w} \tag{7}$$

Substituting the definitions of C_w and P^*_e into Equation 5 results in the following form of P^*_w:

$$P^*_w = \frac{P^*_c}{C_o\left(1 - \frac{\left(1 - \frac{C_m}{C_o}\right)}{4\ Gz\ P^*_{aq}}\right)} + P^*_m \Bigg/ \left(1 + \frac{}{K_m}\right) \tag{8}$$

P^*_{aq} and P^*_w are defined in terms of the intrinsic membrane transport parameters: P^*_c, K_m, and P^*_m. P^*_e, Gz, and C_m/C_o are calculated using experimental parameters (Q, L) and the results of the quantitative analysis of the perfusion samples (C_m, C_o). Nonlinear regression analysis is performed using three equations (equations 3, 7, and 8), two independent variables (C_m/C_o and Gz), and one dependent variable (P^*_e). If mean data is used, a weighting scheme such as 1/standard error or 1/variance should also be employed to account for experimental variability. Example control and data files are available from the corresponding author. After estimates of P^*_c, K_m, and P^*_m are obtained from nonlinear regression analysis, P^*_{aq} and P^*_w are calculated from Equations 3 and 8, respectively. For compounds with a carrier-mediated transport component, the intrinsic transport parameters are calculated first followed by the calculation of intestinal permeabilities. For the MBL method, permeabilities are calculated first. For compounds absorbed by non-concentration dependent processes, passive permeability is calculated directly using either method. The standard deviation (SD) of the NAR-NLR P^*_w at each C_w were calculated using the upper and lower limits of the transport parameters (P^*_c and K_m) from the nonlinear regression. The upper and lower limits of each P^*_w were calculated using equation 8 and the transport parameter values plus or minus one standard deviation, respectively. The mean of these values were taken as the standard deviation of P^*_w. The relative SD was calculated by dividing the estimated SD by the value for P^*_w at each C_w.

Comparisons of MBL and NAR-NLR P^*_w Sensitivity to Changes in C_m/C_o

It is possible to obtain P^*_e values that are close to the calculated P^*_{aq} values for highly absorbed compounds (i.e., where C_m/C_o is low). In these cases, large relative changes in P^*_w are observed for small changes in P^*_e leading to a high degree of variability in the calculated MBL P^*_w. The sensitivity of the MBL or NAR models' P^*_w to changes in P^*_e can be demonstrated by substituting the experimentally observed variable C_m/C_o (fraction of drug unabsorbed) into equation 7 for P^*_e. Taking the derivative of the substituted expression gives the following relationship for the MBL model:

$$\frac{dP^*_w}{d\left(\frac{C_m}{C_o}\right)} = \frac{-4Gz\ P^{*2}_{aq}}{\left[4\ Gz\ P^*_{aq} - \left(1 - \frac{C_m}{C_o}\right)\right]^2} \tag{9}$$

The value of the function in Equation 9 at any value of

C_m/C_o determines the rate of change in P^*_w for small changes in C_m/C_o. Similarly, the first derivative for the NAR-NLR is:

$$-\frac{dP^*_w}{d\left(\frac{C_m}{C_o}\right)} = \frac{4\,P^*_c\,Gz\,P^*_{aq}\,\frac{C_o}{K_m}}{\left(4\,Gz\,P^*_{aq} + \frac{C_o}{K_m}(4\,Gz\,P^*_{aq} + 1) - \frac{C_m}{K_m}\right)^2} \quad (10)$$

In order to determine and contrast the MBL and NAR-NLR models' sensitivities, Gz were selected for each of the three compounds to bracket the experimentally observed low and high values. For formycin B, the low and high values of Gz were 0.029 and 0.048, respectively. For proline, the low and high values of Gz were 0.052 and 0.115. For thymidine, the low and high values of Gz were 0.030 and 0.060. P^*_{aq} were calculated using Equation 3 and 6 for the NAR-NLR and MBL methods, respectively. Equations 9 and 10 and the appropriate transport parameters from Table 4 were used to simulate the changes in P^*_w with respect to changes in C_m/C_o as a function of C_m/C_o. Simulations were performed using Lotus 1-2-3 for Windows.

In order to reduce observed permeability variability, a commonly employed strategy is to restrict the range of experimental C_m/C_o. Therefore, the MBL model sensitivity was also evaluated by maintaining C_m/C_o at a constant value of 0.92. In the simulations, Gz varied from 0.004 to 0.150. Appropriate equations were utilized to calculate P^*_{aq}, P^*_e, and P^*_w. The simulation data were plotted as a function of F, where $F = P^*_{aq}/P^*_w$ or R_w/R_{aq} and R is a resistance.

RESULTS AND DISCUSSION

Validation of the NAR-NLR Method

The cephradine data and results of the MBL analysis were published previously (2). The cephradine SPIP data were selected to validate the NAR-NLR method since the observed range of Gz was narrow (0.031 − 0.043). These values were well within the valid ranges of the MBL ($Gz \geq 0.004$) and NAR ($0.001 \leq Gz \leq 0.5$) analyses. As seen in Table 1, the concentrations studied ranged from 0.01mM to 25mM with the effective permeability decreasing 2.8 fold from the lowest (first order) to highest (zero order) cephradine concentrations. Three to six experiments were performed at each concentration. The data were reanalyzed using the MBL and NAR-NLR methods. The weighting scheme used, 1/(standard deviation (P^*_e)/mean$(P^*_e))^2$, is different from the original analysis (2) resulting in slight differences in the parameter estimates. Three nonlinear regression programs were used to analyze the data: PCNONLIN (version 3, SCI Software, Lexington, KY), BMDP/386 Dynamic (BMDP Statistical Software, Los Angeles, CA) and MINSQ or The Scientist for Windows (MicroMath Scientific Software, Salt Lake City, UT).[5] The permeabilities of cephradine were calculated using the intrinsic membrane transport parameters and the appropriate equations. The calculated P^*_{aq} and P^*_w are given in Table 2. For comparison, the intrinsic intestinal transport parameters were determined using the MBL method, NAR-NLR with Gz constant and NAR-NLR with Gz variable. As seen in Table 3, the carrier permeability (P^*_c), Michaelis constant (K_m), and passive membrane permeability (P^*_m) were not significantly different between the three methods. Mean permeability values in Table 2 and mean intrinsic transport parameters in Table 3 do not vary significantly between the MBL and NAR-NLR methods. Given the narrow range of cephradine Gz, these results were expected and, in terms of mean values, they validate the use of the NAR-NLR method. An advantage of the NAR-NLR method is that the Gz may be treated as a second independent variable during the nonlinear regression analysis. As shown in Table 3 for the cephradine data, the effect of varying Gz was minimal; however, in situations where experimental flow conditions may vary widely for certain drugs, such as in competitive inhibition or concentration dependence studies, this feature may be important.

Table 1. Cephradine Experimental Data Obtained from the Single Pass Intestinal Perfusion Experiment in Rats

C_o	Gz	P^*_e	SD	Wt	n
0.01	0.031	0.84	0.18	22.2	4
0.1	0.033	0.86	0.04	384.5	4
1.0	0.035	0.75	0.17	19.6	5
10.0	0.043	0.28	0.03	113.2	6
25.0	0.038	0.30	0.04	54.9	3

Table 2. Intestinal Wall Permeability of Cephradine Calculated Using the Modified Boundary Layer Solution (Johnson and Amidon, 1988) and the NAR-NLR Method

	Modified Boundary Layer			Numerical Aqueous Resistance		
C_o	P^*_{aq}	C_w	P^*_w	P^*_{aq}	C_w	P^*_w
0.01	2.61	0.0068	1.41	2.74	0.0069	1.31
0.1	2.54	0.066	1.36	2.70	0.068	1.27
1.0	2.54	0.706	1.09	2.65	0.717	1.04
10.0	2.33	8.799	0.33	2.47	8.869	0.32
25.0	2.45	21.90	0.36	2.60	22.08	0.34

Application of NAR-NLR to Formycin B, Proline, and Thymidine Data

The MBL and NAR-NLR analyses were applied to formycin B, proline, and thymidine intestinal perfusion data gener-

Table 3. Comparison of Cephradine Intrinsic Intestinal Transport Parameters Calculated Using the MBL Analysis and the NAR-nonlinear Regression Method with Gz Constant or Variable

	MBL		NAR-NLR: Gz = constant		NAR-NLR: Gz = variable	
Parameter	Estimate	SE	Estimate	SE	Estimate	SE
P^*_c	1.20	0.08	1.12	0.08	1.10	0.09
K_m	1.32	0.81	1.21	0.90	1.27	0.98
P^*_m	0.22	0.09	0.22	0.09	0.22	0.10

[5] Control files used to perform the NAR-NLR analysis are available for each of the three software packages from the corresponding author.

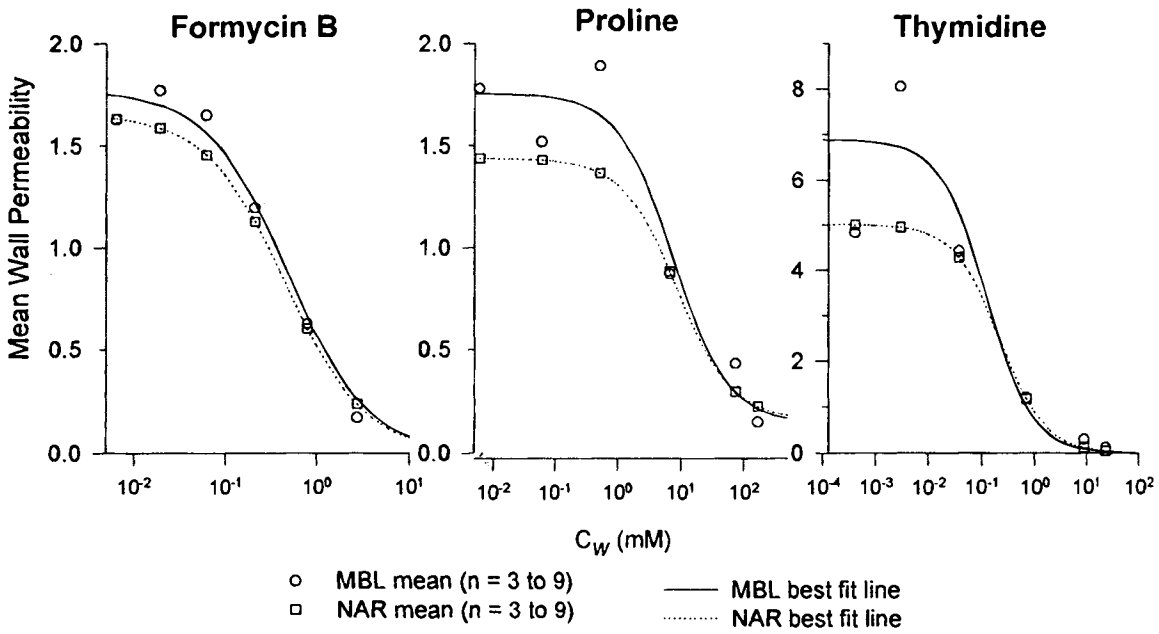

Fig. 1. Plot of the mean, dimensionless wall permeability (P^*_w) versus mean wall concentration of formycin B, proline, and thymidine calculated using the MBL and NAR-NLR methods. The intrinsic transport parameters are listed in Table 4.

ated for this investigation. Gz was treated as an independent variable in the NAR-NLR analysis. Plots of the mean (± SEM) P^*_w versus C_w are shown in Figure 1 for formycin B, proline, and thymidine, respectively, along with the NAR-NLR curve generated from the intrinsic transport parameters and the best fit MBL curve generated from the nonlinear regression of P^*_w and C_w. All NAR-NLR P^*_w fall directly on the fitted line since the P^*_w were calculated using mean intrinsic transport parameters. Table 4 summarizes the intrinsic membrane transport parameters calculated using the MBL and the NAR-NLR analyses. As seen in Table 4, the MBL P^*_e and P^*_m were more variable than the corresponding NAR-NLR values. The MBL Michaelis constant for thymidine was highly variable and not significantly different from zero whereas, when the NAR-NLR method was used, the variability of the K_m value was significantly lower, yielding a reliable parameter estimate. Interestingly, the reliability of the MBL parameter estimates correlated with the magnitude of the difference between the high and low Gz (i.e., flow conditions) used in the SPIP experiments. For cephradine, formycin B, proline, and thymidine, the difference between the low and high Gz values ($\triangle$Gz) were 0.012, 0.019, 0.063, and 0.030, respectively. The two compounds with the highest degree of variability in K_m, proline and thymidine, also had the largest $\triangle$Gz. The uncertainty of the K_m estimate is due to the variability in P^*_w when C_w is approximately less than or equal to K_m since the relative standard errors (RSEs) of P^*_w at concentrations above the K_m are significantly lower. Given the disparity between RSEs above and below the K_m, the MBL and NAR-NLR model-inherent variability were investigated as a function of wall concentration.

Table 4. Comparison of Intrinsic Intestinal Transport Parameters Calculated Using the MBL Analysis and the NAR-NLR Method for Formycin B, Proline, and Thymidine

	MBL		NAR-NLR: Gz = variable	
Parameter	Estimate	SD	Estimate	SD
Formycin B				
P^*_c	1.77	0.06	1.66	0.08
K_m	0.48	0.11	0.45	0.08
P^*_m	0	0	0	0
Proline				
P^*_c	1.61	0.47	1.28	0.13
K_m	7.55	7.14	8.71	4.25
P^*_m	0.14	0.46	0.16	0.09
Thymidine				
P^*_c	6.89	0.90	5.02	0.50
K_m	0.12	0.22	0.22	0.05
P^*_m	0	0	0	0

The P^*_w RSEs for the MBL, NAR-NLR, and ratio of RSEs (RSE_{mbl}/RSE_{nar}) are plotted in Figure 2 for formycin B, proline, and thymidine, respectively. The RSEs of the MBL P^*_w values are disproportionately high at low perfusate concentrations whereas at concentrations above the K_m the RSEs are approximately equal to the RSE_{nar}. The RSEs for the NAR-NLR analysis are relatively constant across all concentrations studied at or below the K_m, however, at higher concentrations, there is an upward trend in RSE. As shown in Figure 2, when the RSE_{mbl} is normalized by the RSE_{nar}, the RSE trend is even more apparent. At low concentrations, the RSE ratio is approximately equal to 4:1 to 6:1. The RSE ratio drops precipitously near the K_m to a value of $\approx$ 1 and remains relatively constant at higher concentrations. This result clearly demonstrates the MBL model-inherent variability at perfusate concentrations below the K_m. The variation using the MBL analysis values could not be explained by a coincident variation in measured P^*_e (not shown) for these compounds. The same trend in variability of MBL P^*_w values at concentrations less than the K_m was

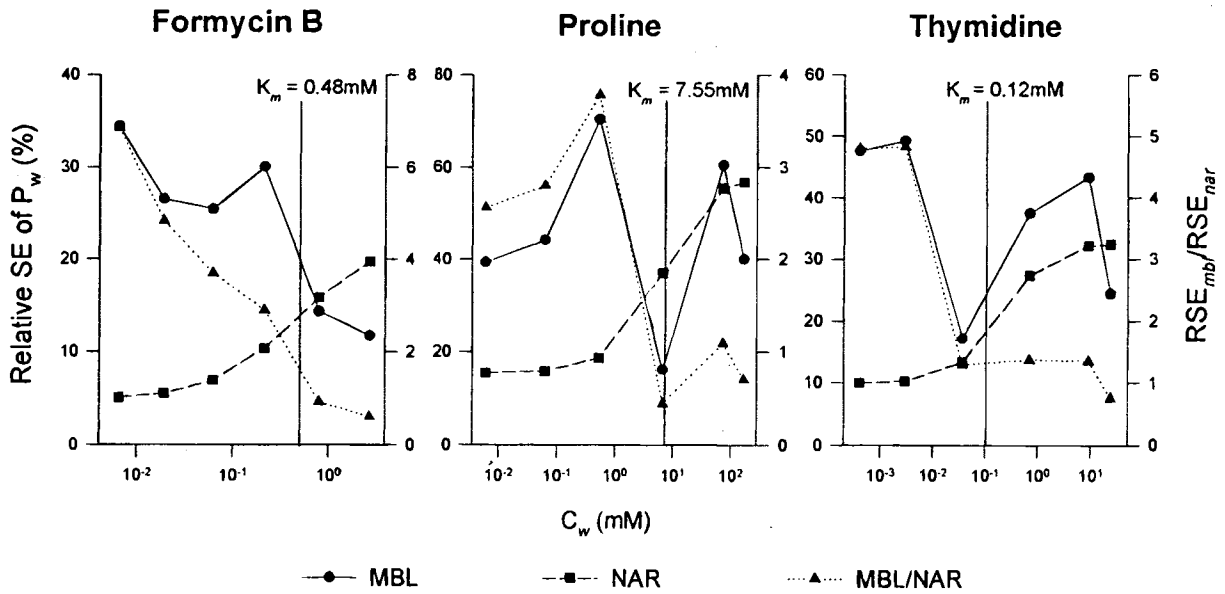

Fig. 2. Plot of the MBL and NAR-NLR relative standard error (RSE) of P^*_w (%) versus mean wall concentration of formycin B, proline, and thymidine and a plot of the ratio the MBL and NAR-NLR RSEs versus mean wall concentration. The vertical lines represent the intrinsic transport K_ms calculated using the MBL method.

observed and previously reported for other carrier-mediated compounds such as cefatrizine, cefadroxil, and cephalexin (2). Since the MBL-inherent variability was greatest at low concentrations (i.e., where C_m/C_o is low and below the K_m), an investigation into the sensitivity of the MBL- and NAR-NLR-P^*_w and intrinsic transport parameter calculations was performed.

Sensitivity of the MBL and NAR-NLR Calculated P^*_w to Changes in C_m/C_o

Since the RSEs at higher concentrations were approximately equal for the MBL and NAR-NLR models, it was hypothesized that the significant increase in RSE_{mbl} at the lower concentrations may be model-dependent. Therefore, using Equations 9 and 10, the model-induced sensitivities of P^*_w to changes in C_m/C_o were investigated for formycin B, proline, and thymidine and the results are shown in Figure 3. The changes in P^*_w with respect to changes in C_m/C_o are plotted as a function of C_m/C_o using Gz values that represent the low and high values observed in the SPIP experiment for each compound. In all cases, the NAR-NLR P^*_w were not sensitive to changes in C_m/C_o as indicated by the nearly horizontal line. The MBL P^*_w, however, demonstrated significant sensitivity to changes in C_m/C_o. For example, the difference in thymidine $dP^*_w/d(C_m/C_o)$, was 10-fold greater at C_m/C_o = 0.80 than at C_m/C_o = 1.00 for the higher Gz values and 50-fold higher at lower Gz values. For the NAR-NLR, the difference was less than 1% at low or high Gz values. Although the MBL variability was significant at both low and high values of Gz, the most significant sensitivity occurred at lower Gz. Therefore, at concentrations below the K_m, where P^*_w is at its maximum, greater variability is expected, consistent with our experimental observations. Above the K_m, it is expected that the NAR-NLR variability should still be less than the variability associated with the MBL analysis; however, the ratio plot in Figure 2 demonstrates that the variability is approximately equal. This is probably due to inherent biological variability.

MBL Model Sensitivity as P^*_w Approaches P^*_{aq}

The preceding discussion and analysis could lead one to believe that the variability of the MBL analysis could be controlled by constraining C_m/C_o to a narrow range or to a constant value (e.g., $C_m/C_o \approx 0.9$) or by holding the intestinal length or flow rate constant. Therefore, in order to generalize the analysis, additional simulations were performed to investigate the sensitivity of P^*_w at a constant C_m/C_o value (C_m/C_o = 0.92). The simulation data were plotted as a function of F (= P^*_{aq}/P^*_w or R_w/R_{aq} where R is a resistance) in Figure 4. Since C_m/C_o was held constant, the only parameter that was varied was Gz. As F approaches 1, i.e., as P^*_w approaches P^*_{aq}, large changes in P^*_w still occur leading to highly variable estimates of P^*_w. Based on these results, it would not be sufficient to focus on constraining C_m/C_o, Q or L but, rather, to examine the relationship between P^*_{aq} and P^*_w and to select the data that matched the restrictions of the MBL analysis (i.e., to disregard permeability values when variability was high). In a practical sense, it would be highly inefficient and resource intensive to select only those studies where the perfusion results matched these highly constrained conditions. The maximum P^*_w would have to be calculated in order to determine the F value for that particular experiment. As seen in Figure 4, if the F value is less than three or four, the variability of the P^*_w calculation is expected to be high. For proline for example, the P^*_{aq} at low and high Gz is equal to 1.46 and 2.13, respectively. Therefore, the greatest values of P^*_w that would still have reasonable variability (i.e., F = 4) are 0.37 and 0.53 for the low and high Gz, respectively. For the less conservative F = 3, the values become 0.49 and 0.70, respectively. These calculations indicate, however, that P^*_w above these values will be highly variable. This theoretical

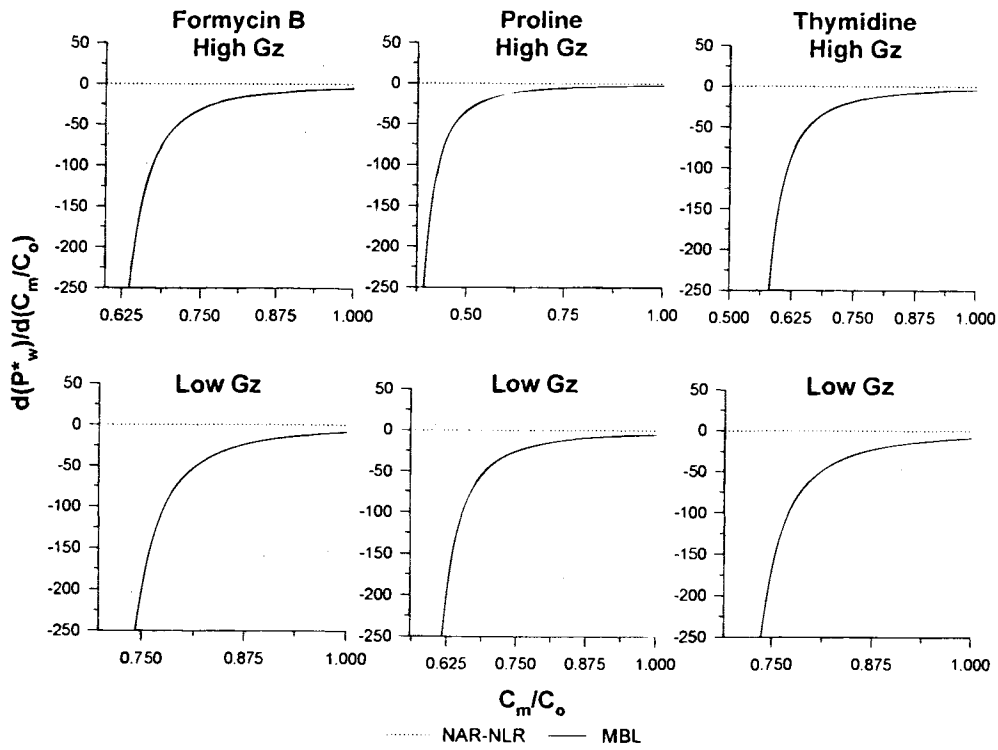

Fig. 3. A plot of the MBL- and NAR-NLR-model sensitivity of P^*_w with respect to C_m/C_o versus C_m/C_o for formycin B, proline, and thymidine, respectively. The top panels represent the simulations at the highest experimentally observed Gz values for each compound whereas the lower panel represents the simulations at the lowest experimentally observed Gz values.

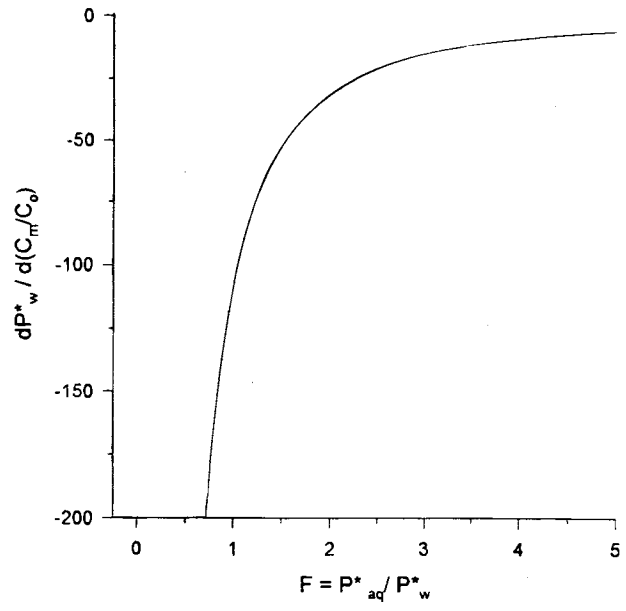

Fig. 4. A plot of the MBL-model sensitivity of P^*_w with respect to C_m/C_o versus F (P^*_{aq}/P^*_w) over a range of Gz = 0.004 to 0.150 with C_m/C_o = 0.92.

observation is confirmed by the current data. The NAR-NLR analysis would allow for the use of all experimentally-derived data since it offers the widest range of experimental flow conditions. By examining the general relationship between P^*_w and P^*_{aq}, the MBL-model induced sensitivity has been shown to apply to any compound, independent of absorption mechanism when P^*_w approaches P^*_{aq}. Furthermore, the NAR-NLR methodology facilitates the analysis of intestinal perfusion data for compounds absorbed by carrier-mediated pathways.

CONCLUSIONS

In summary, the NAR-NLR analysis allows for the direct calculation of the intrinsic membrane transport parameters from the experimental perfusion data and facilitates the analysis of perfusion data for compounds with high intestinal wall permability. The NAR-NLR analysis allows for the use of a wider range of experimental flow conditions than the MBL analysis while providing more reliable estimates of intrinsic transport parameters and P^*_w. The NAR-NLR analysis is also shown to reduce the variability of parameter estimates for any compound, independent of absorption mechanism when P^*_w approaches P^*_{aq}.

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