Unbiased Membrane Permeability Parameters for Gabapentin Using Boundary Layer Approach

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ABSTRACT

The present study was performed to determine the relative contribution of both passive and nonpassive transport processes in jejunal absorption of gabapentin. The oral absorption of gabapentin was studied using in situ single pass intestinal perfusion technique in fasted rats. Unbiased intrinsic membrane absorption parameters such as maximal flux, Michaelis constant, carrier permeability, and membrane permeability were calculated using a modified boundary layer model. Gabapentin intestinal perfusion results indicate that its jejunal absorption in rats occurs via a nonpassive process, with no significant passive absorption component, as demonstrated by saturable absorption kinetics and its concentration-dependent permeability. A good correlation ($r^2 = 0.88$) between observed human absorption fraction and calculated (from in situ rat intestine) human absorption fraction was obtained.

KEYWORDS: carrier-mediated transport, concentration dependent, gabapentin, intestinal perfusion, permeability

INTRODUCTION

Gabapentin, [(1-aminomethyl) cyclohexane acetic acid] is an antiepileptic drug, related structurally to γ -aminobutyric acid (GABA), in which the GABA analog portion is incorporated into a cyclohexane ring to allow its easy transport across the blood-brain barrier.¹ Gabapentin is highly water soluble, with an octanol/buffer (pH 7.4) log P value of -1.10 , and is zwitterionic at physiological pH (pK_a value of 3.68 and 10.7).² Absolute bioavailability of gabapentin is dose dependent, and the plasma concentration of the drug is therefore not directly proportional to dose. Based

on the data from several pharmacokinetic studies in healthy subjects, absolute bioavailability was found to be $57\% \pm 9.6\%$ and $42\% \pm 10\%$ following single doses of 300 and 600 mg, respectively.^{3,4}

The absorption pathway of gabapentin initially was characterized as passive diffusion,⁵ and carrier-mediated transport mechanism was ruled out based on an apparent linear dependence of absorption rate with concentration. However, the study had only few data in the range of 1 to 10 mM, where a sharp decrease in fraction of dose absorbed occurs. In a later study, Stewart et al.,⁶ using single pass intestinal perfusion experiments, postulated the existence of a saturable transport process on the basis of concentration-dependent and competitive inhibition study in rat intestine. A 70% to 75% decrease in permeability was observed when the concentration of gabapentin was increased from 0.01 to 50 mM in situ. The same study, using an everted rat intestinal ring system reported inhibition of gabapentin uptake by leucine and phenylalanine. Its structural resemblance to amino acids suggested the involvement of amino acid transporter in absorption. L system transporter is the major transport system for gabapentin in astrocytes, synaptosomes, and CHO cells.⁷ Unlike the amino acids, the amino group in gabapentin is γ rather than α to carboxyl group. However, low energy conformation of gabapentin resembles an L form of large neutral α -amino acids. The conformational effects enforced by the β -cyclic side chain makes it possible to react with L transporters. However, choice of an optimum permeability enhancer cannot be made without a clear understanding of the mechanisms involved in its oral absorption.

In general, for any molecule possessing both carrier and passive transport, it is nonspecific passive transport that can be exploited for absorption enhancement more effectively. Most of hydrophilic, zwitterionic,⁸ β -lactam penicillins⁹ and cephalosporins,¹⁰ as expected, do not possess a passive transport component. However, amoxicillin has exceptionally high passive transport,⁹ which obviously cannot be attributed to a low-capacity paracellular transport. Therefore, actual contribution of either transport process for any molecule cannot be accurately determined without actually performing experimentation.

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An absolute bioavailability of $\sim 50\%$ makes gabapentin a good candidate for improvement of oral bioavailability. However, choice of an optimum permeability enhancer cannot be made without a clear understanding of the mechanisms involved in its oral absorption. The present study was thus aimed to determine the relative contributions of both passive and nonpassive pathways in intestinal absorption of gabapentin. In the highly charged state (zwitter-ionic), contribution of passive transport is expected to be low and by paracellular pathway, if any. The mechanism of absorption of gabapentin was investigated using an *in situ* rat perfusion method at different concentration levels (3, 5, 10, and 40 mM). The intrinsic absorption parameters were obtained by boundary layer analysis.¹¹ A correlation between predicted human fraction absorbed and observed fraction absorbed for gabapentin has also been determined using a macroscopic mass balance approach for the complete radial mixing model.¹²

MATERIALS AND METHODS

Materials

Gabapentin was a gift from Ranbaxy Laboratories Ltd (Gurgaon, India). Phenol red was obtained from Ranbaxy Laboratories Ltd (S.A.S. Nagar, India). Sodium chloride, potassium chloride, potassium di-hydrogen phosphate, and orthophosphoric acid were purchased from Loba Chemie Private Ltd (Mumbai, India). Di-sodium hydrogen phosphate was obtained from the Central Drug House Private Ltd (New Delhi, India). Di-potassium hydrogen phosphate was supplied by S.D. Fine Chemicals Ltd (Mumbai, India). Urethane was obtained from the Himedia Lab Ltd (Mumbai, India). Acetonitrile was obtained from J.T. Baker Chemical Co (Mexico City, Mexico). All chemicals were of analytical or high-performance liquid chromatography (HPLC) grade and were used as received.

Perfusion Solutions

Blank Perfusion Solution

Perfusion solution containing 138 mM NaCl, 2.7 mM KCl, 8.1 mM Na_2HPO_4 , and 1.5 mM KH_2PO_4 was adjusted to pH 6.5 ± 0.02 using a pH meter (CyberScan PH 510, Eutech Instruments, Singapore) with dilute orthophosphoric acid.

Perfusion Drug Solution

An appropriate amount of gabapentin (3, 5, 10, or 40 mM) and phenol red (50 mg/L) was added to blank perfusion buffer, and osmolality was adjusted to 300 ± 10 mOsm/kg with sodium chloride, using a vapor pressure osmometer (VAPOR, model 5520, Wescor, Logan, UT).

Stability Study

The stability of gabapentin was tested by incubation in perfusion solution and blank perfusate from rat intestines at $37^\circ\text{C} \pm 1^\circ\text{C}$ for 5 hours in blank perfusate. Samples were withdrawn at time intervals of 0, 1, 2, 3, 4, and 5 hours. Samples were immediately analyzed by HPLC method. The blank perfusate was obtained by passing the blank perfusion solution through a segment of intestine *in situ* for 1 hour at a flow rate of 0.2 mL/min.

In Situ Single Pass Perfusion

Approval for the study was granted by the Institutional Animal Ethical Committee (IAEC/01/079). The method used has been reported previously.⁹ Male Sprague Dawley rats weighing 220 to 300 g were fasted for 12 to 20 hours prior to each experiment. Water was given *ad libitum*. Rats were anesthetized by intraperitoneal injection of 50% wt/vol urethane solution (1.6 g/kg). The abdomen was opened with a midline incision and the small intestine was taken out carefully to avoid disturbance to the circulatory system. The proximal part of the jejunum, 2 to 5 cm below the ligament of Trietz was cannulated with a glass cannula and connected to a single-channel peristaltic pump (P-1, Pharmacia Biotech, Uppsala, Sweden). A second incision, ~ 10 to 15 cm distal to first incision, was made and connected with an outflow glass cannula (L shape). The intestine segment was perfused with blank perfusion buffer ($37^\circ\text{C} \pm 1^\circ\text{C}$) until perfusate was clear. The intestine was subsequently perfused with drug solution maintained at $37^\circ\text{C} \pm 1^\circ\text{C}$ at a perfusion rate of 0.2 mL/min. During the experiment rats were kept under a heating lamp, and the exposed abdomen was covered with a cotton pad (wetted with normal saline) and parafilm (American National Can Co, Neenah, WI) to minimize dehydration. Steady state was achieved within ~ 30 minutes after which 4 to 5 samples were taken at regular intervals of 15 minutes. After taking the last sample, the length of the intestine was measured by placing a piece of thread along the intestine and measuring the thread with a ruler. The study was performed on 4 to 5 animals for each concentration level.

Analytical Method

Inlet and outlet samples from perfusion experiments were analyzed for gabapentin and phenol red, and stability samples were analyzed for gabapentin using HPLC (Class 10 AVP, Shimadzu, Tokyo, Japan) consisting of an auto injector (SIL-10 ADVP), a solvent pump (SCL-10 AVP), and a UV-visible detector (SPD-10 AVP) and a reversed phase column (LichroCART, RP-8, 250×4 mm, Lichrospher 5 micrometers, Merck, Germany).

Gabapentin Assay

HPLC analysis of gabapentin was performed using a mobile phase, buffer 93%, (1.2 g/mL of K₂HPO₄, pH adjusted to 6.9 with orthophosphoric acid) and 7% acetonitrile: flow rate, 1 mL/min; detector wavelength, 272 nm; retention time, ~6 minutes; injection volume, 20 µL. The method was previously validated for various parameters in the range of 100 to 2000 µg/mL gabapentin ($r^2 = 0.9999$). The accuracy and precision in terms of coefficient of variation were 1.19% and 1.74%, respectively.

Phenol Red Assay

Phenol red analysis was performed using a mobile phase, water (75%) and acetonitrile (25%): flow rate, 0.8 mL/min; detector wavelength, 435 nm; retention time ~2.5 minutes; and injection volume, 5µL. The method was found to be linear in the range of 10 to 100 µg/mL of phenol red ($r^2 = 0.9999$). The coefficients of variation for accuracy and precision were 1.76% and 1.23%, respectively.

Data Analysis

Water Flux Corrections

Assuming that phenol red will not be absorbed from the intestine, any change that occurs in perfusate drug concentration due to water absorption or secretion (water flux) was corrected by using the following expression.¹³

$$C_m = C'_m \cdot CPR_{in}/CPR_{out} \quad (1)$$

The percentage of water transport per centimeter perfused intestinal length (%WT/cm) for each sample was calculated from

$$\%WT/cm = [(CPR_{in} - CPR_{out})/CPR_{out}] \cdot 100/L \quad (2)$$

Where C_m and C'_m represent the uncorrected and corrected (for water flux) drug concentration in perfusate, respectively. CPR_{in} and CPR_{out} represent the phenol red concentration in perfusion (inlet) and perfusate (outlet) solution, respectively, and L is length of perfused intestine.

Estimation of Intrinsic Absorption Parameters

Modified boundary layer analysis^{11,14} was applied to experimental data for estimation of the dimensionless absorption parameters; namely, effective permeability (P_{eff}^*), aqueous permeability (P_{aq}^*), wall permeability (P_w^*), and carrier permeability (P_c^*).

$$P_{eff}^* = (1 - C_m/C_o)/4.Gz, \quad (3)$$

where C_m and C_o are the outlet perfusate concentration (corrected for water flux) and inlet perfusion solution concentration, respectively, and Gz is Graetz number (see Equation 5).

$$P_{aq}^* = (A.Gz^{1/3})^{-1} \quad (4)$$

Graetz number (Gz) is calculated from the following expression:

$$Gz = \pi DL/2.Q, \quad (5)$$

where D , L , Q are the diffusion coefficient of gabapentin, length of intestine, and inlet perfusion flow rate, respectively.

Unit less quantity, A (in Equation 4), was approximated using the following:

$$0.004 \leq Gz \leq 0.01; A = 10.0 Gz + 1.01$$

$$0.01 \leq Gz \leq 0.03; A = 4.5 Gz + 1.065$$

$$0.03 \leq Gz; A = 2.5 Gz + 1.125$$

$$P_w^* = P_{eff}^*/(1 - P_{eff}^*/P_{aq}^*) \quad (6)$$

The wall concentration, C_w , was calculated by

$$C_w = C_o(1 - P_{eff}^*/P_{aq}^*). \quad (7)$$

For a combination of carrier and passive uptake mechanisms the wall permeability was assumed to be of the following form:

$$P_w^* = J_{max}^*/(K_m + C_w) + P_m^*, \quad (8)$$

where J_{max}^* is maximal flux, K_m is the intrinsic Michaelis constant, and P_m^* is intrinsic passive membrane permeability.

The dimensionless carrier permeability (P_c^*) is defined as

$$P_c^* = J_{max}^*/K_m. \quad (9)$$

Rewriting Equation (9) in terms of carrier permeability results in

$$P_w^* = P_c^*/[1 + C_w/K_m] + P_m^*. \quad (10)$$

Figure 1. Percentage water transport per centimeter perfused intestine length for a perfusion experiment.

Estimation of Aqueous Diffusion Coefficient

The diffusion coefficient (D) used in permeability evaluation (in Equation 5) was calculated according to the Hayduk-Laudie expression.¹⁵

$$D = 13.26 \times 10^{-5} \eta^{-1.4} V_a^{-0.589}, \quad (11)$$

where η denotes the viscosity of water (0.6915 cP at 37°C), V_a is the solute molal volume at normal boiling point ($\text{cm}^3/\text{g/mol}$), and D denotes the binary diffusion coefficient at infinite dilution (cm^2/s). Molal volume (V_a) for gabapentin was estimated using Le Bas additive increment method.

Calculation of Fraction Dose Absorbed in Humans

The fraction dose absorbed in humans using parameters determined from perfusion experimental method was calculated using complete radial mixing model.¹²

$$F = 1 - \exp(-2.An). \quad (12)$$

Dimensionless absorption number (An) is defined as ratio of the fundamental mass transfer processes affecting drug removal from the intestine: absorption and convection.

$$An = SF(P_w^* \cdot Gz), \quad (13)$$

where SF is scaling factor = 1.27 for complete radial mixing model. For compounds that are absorbed by a carrier-mediated process, concentration-dependent permeability requires the use of mean permeability for these predicted human fraction absorbed calculations.¹⁶ The mean permeability is defined as follows:

$$\bar{P}_w^* = \frac{\int_{C_0}^0 P_w^* dc}{\int_{C_0}^0 dc}, \quad (14)$$

where $C_o = D_o/V_L$, D_o is the dose administered, and V_L is the luminal volume (also taken to be volume given with dose = 250 mL). Substituting Equation 10 into Equation 14 and integrating gives

$$\bar{P}_w^* = P_m^* + P_c^* K_m/C_0 \ln(1 + C_0/K_m). \quad (15)$$

RESULTS AND DISCUSSION

There was no significant change in gabapentin concentration at 37°C for 5 hours in rat intestinal blank perfusate. Gabapentin was found to be stable during the experimental conditions. The values of V_a (by Lebas additive increment method) and D for gabapentin (from Equation 11) were found to be $216 \text{ cm}^3/\text{mol}$ and $9.38 \times 10^{-6} \text{ cm}^2/\text{s}$, respectively. The permeability values were calculated from 3 to 5 steady-state concentration of gabapentin. Steady state was considered to have been achieved when the concentration of gabapentin and phenol red in the perfusate was stable (~ 30 -45 minutes). Figures 1 and 2 represent the water transport (as calculated from Equation 2) and ratio of corrected outlet concentration and inlet concentration of gabapentin (at $C_o = 40 \text{ mM}$) as a function of time during the experiment. The transmembrane water flow across intestinal membrane influences the permeability of hydrophilic drugs.^{17,18} Water transport below 0.5%/cm of perfused intestine length was considered normal, and experiments with higher water transport were not used in determination of membrane parameters. The flow rate of 0.1 mL/min and longer intestine segments ($\sim 18 \text{ cm}$) were used at 40 mM, whereas, the flow rate of 0.2 mL/min and smaller intestinal segments (10-12 cm) were used for gabapentin concentrations lower than 40 mM to change C_m/C_o to at least 0.95 to allow a significant analytical concentration difference

Figure 2. Ratio of corrected outlet concentration and inlet concentration of gabapentin at bulk concentration of 40 mM.

Table 1. Summary of the Intrinsic Membrane Parameters Derived From the Nonlinear Regression Using the Model of Wall Permeability in Equations 8 and 10*

Absorption Parameter	J_{max}^* (mM)	K_m (mM)	P_c^*	P_m^*
Value (SEM)	4.862 (0.88)	11.09 (2.82)	0.442 (0.038)	ND 0

*ND 0 indicates not statistically different from zero.

between C_m and C_o , but not more than 0.85 to prevent aqueous resistance dominance over membrane resistance.¹¹

A previous study had established existence of a saturable transport mechanism in the intestinal absorption of gabapentin as the underlying cause for lack of dose proportionality.⁶ The authors used an in situ model of single pass intestinal perfusion in rats for elucidating the absorption mechanism. However, determination of membrane transport parameters from intestinal perfusion experiment may be “unreliable” due to bias introduced owing to contribution of perfusional flow rate. This problem has been tackled by proposing a film model for the perfusion experiment,¹⁰ using a numerical method and approximation of boundary layer.¹¹ The permeability results of the perfusion experiment were fitted to Equations 7 and 9 with weighted nonlinear regression using statistics software SigmaStat. The data were weighted by reciprocal variance of P_w^* . All data were initially fit to the 3-parameter model (J_{max}^* or P_c^* , K_m and P_m^*). However, the value of P_m^* was not significantly different from zero, thus reducing the model to 2 parameters (J_{max}^* or P_c^* and K_m). The intrinsic absorption parameters are enlisted in Table 1. Figure 3 shows the fitted curve.

Intrinsic wall permeability of gabapentin was found to be concentration dependent, as reported previously,⁶ using in situ single-pass intestinal perfusion model. A dose-dependent absorption occurs when there is some rate-limiting step to drug absorption (eg, limited solubility, nonmonoexponential gastric emptying, carrier-mediated transport or metabolism [luminal, gutwall, or first pass]). Gabapentin is highly water soluble and does not metabolize in humans and rats¹⁹; and the in situ rat intestine perfusion model lacks the complexity of gastric emptying. Hence, a concentration-dependent absorption of gabapentin in “in situ rat intestinal model” can be attributed to carrier transport only. Therefore, no inhibition study was performed to confirm the existence of a carrier transport system.

Dose-dependent absorption due to carrier transport process may or may not have a simultaneous passive transport component, as both transport processes are independent. The value of P_m for gabapentin is significantly not different from zero as calculated from nonlinear fit (Equations 8 and 10). Since passive diffusion is a function of polarity and gabapentin exists as a zwitterionic molecule at jejunal

pH, its passive diffusion is expected to be low. On the other hand, amino acids phenylalanine and leucine, expected to share same carrier transport,⁶ also possess non-significant passive diffusion.¹⁴ Despite its configurational similarity to leucine, gabapentin has lower P_c^* than the amino acid,¹⁰ suggesting that gabapentin either is transported partly by amino acid carrier system or has lower affinity for the system transporters than the amino acid. K_m represents a complex binding coefficient that may or may not be heterogeneous.

Drug permeability is one of the fundamental parameters in drug absorption,²⁰ and in the absence of any solubility or metabolism problem, rat in situ intestine permeability value unlike physicochemical properties can be used to predict drug absorption, independent of drug structural class or mechanism.^{12,16} It is understood that physicochemical properties of a drug cannot predict its affinity for a specific carrier, and sometimes a minor structural change in a molecule may result in a drastic change in carrier specificity; yet, a significant difference in passive diffusion of analogs aminopenicillins⁹ is always difficult to attribute to a physicochemical parameter. Absorption measurements in in situ models have been successfully compared with human absorption in previous studies.^{11,15}

The gabapentin fraction absorbed in humans was predicted from rat intestinal permeability and successfully compared with observed fraction absorbed using macroscopic mass balance approach for complete radial mixing model. A

Figure 3. Plot of the intrinsic wall permeability versus the wall concentration of gabapentin. Permeabilities are plotted as mean and error bar represents the SEM. Solid line shows the best fit line.

Table 2. Summary of Gabapentin Pharmacokinetics and Predicted Fraction Absorbed Calculation*

Gabapentin Dose (mg)	Gabapentin Dose [†] (mM) C_0	$\overline{P}_w^*$	An	Fraction Absorbed	
				Predicted [‡]	Observed [§]
100	2.3	0.402	0.510	63.9	73.8
300	7.0	0.343	0.435	58.1	68
600	14.0	0.286	0.363	51.6	41.7
800	18.7	0.259	0.329	48.2	41.7
900	21.0	0.248	0.315	46.7	43.3
1200	28.1	0.220	0.280	42.8	35.7
1600	37.4	0.193	0.246	38.8	35.7

* $An = SF (P_w^* \dots Gz)$. SF is scaling factor = 1.27 for complete radial mixing model; $Gz = 1$ and $\overline{P}_w^*$ mean wall permeability is calculated from Equation 15.

[†]Calculated assuming human intestinal volume for dose dissolution is 250 mL (molecular weight of gabapentin is 171 Da).

[‡]The estimated fraction dose absorbed from the macroscopic mass balance analysis using the complete radial mixing model $[1 - \exp(-2 \cdot An)]$.¹²

[§]Data from Stewart et al.⁶

good correlation ($r^2 = 0.88$) was obtained between predicted fraction observed and observed fraction absorbed for gabapentin (Table 2) using macroscopic mass balance approach for complete radial mixing model. A close correlation between the predicted and observed fraction of gabapentin absorbed in humans establishes suitability of this study for mimicking human absorption.

For the drugs in which dissolution and metabolism are not of significant concern, simple Equation 12 using wall permeability values may be very useful to estimate the human oral availability of drugs irrespective of transport process. The carrier permeability is concentration dependent, and uptake of drug from a finite volume element will increase with decrease in concentration; therefore, use of intrinsic wall permeability (P_w^*) in Equation 12 results in underestimation of fraction absorbed. Therefore, absorption measurement in such a case shall include a kind of mean permeability over the range of concentration involved. Equation 14 is an approximation to mean permeability and is comparable to first approximation by numerical solution.²¹ The mean wall permeability is higher than intrinsic wall permeability, and therefore predicted fraction absorbed values are higher and more logical in latter case. In many cases, the first approximation may be sufficiently accurate, but successive better approximation can be obtained from a numerical solution as in microscopic simulation.²²

CONCLUSION

In conclusion, the study establishes existence of a carrier-mediated transport and lack of passive diffusion for gabapentin, thus allowing development of strategies for improving its absorption. Further, it also simulates the prediction of human fraction absorbed from rat intestine permeability data.

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