Human Jejunal Permeability Predicted from Caco-2 Assay – a Biophysical Model Applied to Fluoroquinolone Antimicrobials

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INTRODUCTION

Since the introduction of nalidixic acid in the 1960s, many quinolone derivatives have been synthesized and analyzed for bacteriostatic antiseptic activity (e.g., in the treatment of urinary infections). The second-generation fluoroquinolones, such as ciprofloxacin, have proven to be very useful broad range therapeutic agents. Most quinolones are orally administered, which is an important advantage over parenteral antibiotics. Considerable progress in predicting oral fraction absorbed of fluoroquinolones has been made, based on measurements of properties such as permeability, obtained *in vitro* using the Caco-2 assay or *in situ* intestinal perfusion in rat. Even though uptake/efflux carried-mediated processes have been reported for some quinolones using Caco-2 and/or *in situ* animal models, the *in vivo* predominant absorption mechanism is the passive absorption pathway.[1,2]

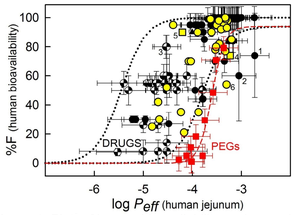


Figure 1. Plot[3] of human bioavailability vs. human jejunal permeability data from Lennernäs's and Amidon's groups.

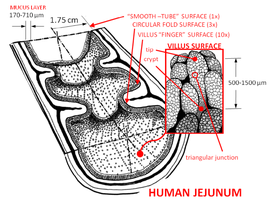


Figure 2. A representation of the structure of the human jejunum. [3]

A recently-reported[3-5] biophysical model for predicting human absorption, based on the published human jejunal permeability data (Fig. 1), reconciled with the dimensions of the small intestine (Fig. 2), was correlated with measured Caco-2 permeability.

The objective of the present study was to predict human jejunal permeability, P_{eff}, and absorption, %Fa, for a series of 18 fluoroquinolones, using the new biophysical model based on measured Caco-2 permeability.[3] Values of P_{eff} have not been reported for these compounds, and not all of the compounds have reported human absorption values.

BIOPHYSICAL MODEL PREDICTION METHOD

Caco-2 apparent permeability values, P_{app}, were collated from various literature sources, and pre-treated with the program, *p*CEL-X (*p*ION), to obtain transcellular permeability values at pH 6.5, $P_c^{6.5}$. This involved removing the effect of paracellular (*para*) and aqueous boundary layer (*ABL*) permeability contributions from the apparent Caco-2 values, and adjusting the resultant transcellular (*trans*) values to the pH of interest using pK_a values of the quinolones. The model equation used

$$\frac{1}{P_{eff}} = \left(\frac{1}{P_{eff}^{ABL}} + \frac{1}{P_{eff}^{trans} + P_{eff}^{para}} \right) = \frac{1}{k_{VF}} \cdot \left(\frac{h_{ABL}}{D_{aq}} + \frac{1}{P_c^{6.5} + P_{para}} \right) \qquad (1)$$

where h_{ABL} = ABL thickness in the *in situ* jejunal perfusion experiment, D_{aq} = diffusivity of the drug, P_{para} = paracellular permeability, and k_{VF} is the jejunal surface area expansion factor (cf., Table 1).[3]

As a minor extension to the Adson *et al.*[6] paracellular model, it was assumed that there exist two populations of junctional pores: (a) high-capacity porosity-pathlength, ε/δ, size-restricted and cation-selective pathways, and (b) secondary ε/δ_2 low-capacity, size- and charge-independent pathways (cf., Table 1). This dual-pore population paracellular equation is

$$P_{para} = \frac{\varepsilon}{\delta} \cdot D_{aq} \cdot F\left(\frac{r_{HYD}}{R} \right) \cdot E(\Delta\varphi) + \frac{\varepsilon}{\delta_2} \cdot D_{aq} \qquad (2)$$

where E(Δφ) is an electric field function (with values of 0.78-1.26 for the drugs) and F is the Renkin pore sieving equation (with values of 0.053-0.096 for the drugs).[3] The parameters used in this study are summarized in Table 1.

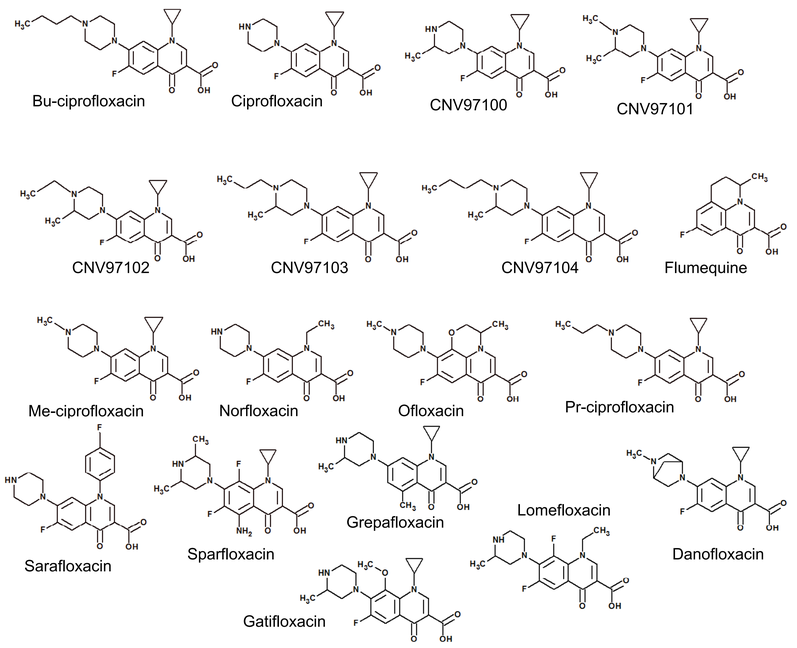
With predicted P_{eff}, a standard exponential function was used to determine values of %Fa, with small intestine transit time, t, set to 2.7 h (best fit).

$$\%F_a = \left(1 - e^{-\left(\frac{2 \cdot 3600}{1.75} \cdot P_{eff} \cdot t\right)} \right) \cdot 100\% \qquad (3)$$

TABLE 1. BIOPHYSICAL HUMAN P_{eff} MODEL PARAMETERS USED[3]

k_{VF}	R(Å)	ε/δ (cm^{-1})	ε/δ_2 (cm^{-1})	Δϕ (mV)	h_{ABL} (μm)
33.5	11.2	0.53	0.027	-30.6	500

RESULTS AND DISCUSSIONS



The predicted P_{eff} (Table 2, in 10^{-4} cm/s units) ranged from 0.6 (norfloxacin) to 4.6 (N-butylciprofloxacin) and 5.6 (flumequine). Most of the fluoroquinolones are ABL-limited in their permeation. The calculated %Fa for norfloxacin = 48% (exp. 35%) and for ciprofloxacin = 68% (exp. 70-85%). Most of the fluoroquinolones had calc %Fa > 90%, and thus are expected to be well absorbed. The three least-absorbed were norfloxacin, ciprofloxacin, and sarafloxacin. Ciprofloxacin showed three passive mechanisms of permeation: transcellular = 69%, ABL-limited transcellular = 19% and paracellular = 12% (cf., Table 2). Norfloxacin was predicted to have the highest paracellular route = 26%, with ABL = 11% and transcellular = 63%.

TABLE 2. PREDICTED HUMAN JEJUNAL P_{eff} AND HUMAN ABSORPTION

DRUG	log P_o Caco-2	$P_c^{6.5}$	pK_a		P_{eff} (10^{-4} cm/s)	% para	% ABL	% trans	%Fa (obs)	Fa% (calc)
Bu-ciprofloxacin	-3.35	308	8.00	6.12	**4.6**	0	96	4		**99**
Ciprofloxacin	-5.34	3.1	8.62	6.16	**1.0**	12	19	69	70-85	**66**
CNV97100	-5.06	6.7	8.53	5.95	**1.6**	4	32	63		**84**
CNV97101	-4.06	63	7.74	6.01	**4.0**	0	81	19		**99**
CNV97102	-4.41	26	8.41	6.18	**3.1**	1	65	35		**97**
CNV97103	-4.48	22	8.30	6.21	**2.9**	1	61	38		**96**
CNV97104	-4.44	23	8.37	6.26	**2.9**	1	62	37		**96**
Flumequine	-2.80	873	6.59		**5.6**	0	98	2		**100**
Me-ciprofloxacin	-3.62	154	7.83	6.21	**4.6**	0	91	9		**99**
Norfloxacin	-5.67	1.3	8.63	6.26	**0.6**	26	11	63	35	**47**
Ofloxacin	-4.75	14.2	8.59	5.89	**2.5**	2	47	51	90	**92**
Pr-ciprofloxacin	-2.70	1472	7.79	5.98	**4.8**	0	99	1		**100**
Sarafloxacin	-5.25	4.4	8.66	5.92	**1.2**	6	25	68		**74**
Sparfloxacin	-4.69	15	8.35	6.05	**2.5**	1	54	45	92	**94**
Grepafloxacin	-4.68	15	8.7	6.1	**2.6**	2	50	48	96	**94**
Levofloxacin	-4.29	41	8.59	5.89	**3.6**	0	74	26	99-100	**98**
Lomefloxacin	-4.47	24	8.7	6.1	**3.1**	0	68	32	95-98	**98**
Danofloxacin	-5.18	4.6	8.7	6.1	**1.3**	7	26	68		**75**
Gatifloxacin	-4.99	7.3	8.8	6.1	**1.7**	4	35	61	96	**85**

CONCLUSIONS

Estimates of P_{eff} can be predicted by the biophysical model, starting from measured Caco-2 permeability values at pH 6.5. From these P_{eff} values, the human absorption was calculated. Where absorption comparisons were possible, the agreement was quite good.

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