Predicting the human jejunal permeability and fraction absorbed of fluoroquinolones based on a biophysical model

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Abstract. The purpose of this study is to predict human jejunal permeability (P_{eff}) and fraction absorbed in human (%F_a) for a group of antibacterial fluoroquinolones (FQs), by using a biophysical model based on measured Caco-2 permeability. The predicted P_{eff} (in 10^{-4} cm·s^{-1} units) ranged from 0.7 (norfloxacin) to 4.5 (pefloxacin). The calculated %F_a for norfloxacin = 51% (lit. 35%) and for ciprofloxacin = 76% (lit. 81%). Most of the FQs showed calculated %F_a > 90%, and are expected to be well-absorbed. Estimates of P_{eff} can be predicted by the biophysical model. From these values, the human absorption may be calculated. Where absorption comparisons were possible, the agreement was acceptably good.

Keywords: permeability, fraction absorbed, fluoroquinolones, human intestine, Caco-2

1. Introduction

Fluoroquinolones (FQs) are among the most often prescribed antimicrobial agents, with a broad anti-infectious spectrum against a number of both Gram-positive and Gram-negative bacteria [1]. The development of FQs was originated with nalidixic acid [2]. The drug action of FQs involves the inhibition two enzymes which are required for bacterial DNA synthesis, namely, topoisomerase II (DNA gyrase) and/or topoisomerase IV. The process is believed to involve the formation of a ternary complex consisting of drug, DNA, and the enzyme that interferes with DNA transcription, and subsequently leading to rapid bacterial cell death [3]. This has been demonstrated by a structural study using a subdomain of topoisomerase derived from Streptococcus pneumoniae and two FQs, moxifloxacin and clinafloxacin [4].

It has been recognized that there is a continuing need for new antimicrobial agents to deal with emerging bacterial infections [5,6]. Effective antibacterial treatment requires not only a suitable antibiotic, but also the administration of an appropriate dosing regimen (dose and schedule) to maximize the clinical benefits [7]. The optimal dose drives the drug exposure (i.e. pharmacokinetics), which is correlated to the pharmacodynamic effect of the antimicrobial agent [8]. Clearly, the ability

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of predicting the pharmacokinetic behavior in humans and/or animals would be useful in the course of developing antimicrobial agents.

Prior to pharmacokinetic study and in vivo efficacy study, it is generally desirable to assess the suitability of the molecules in more bio-relevant models. The rat gut in situ absorption model has been applied for this purpose [9]. While a good correlation between drug intestinal permeability of human and rat small intestine has been reported [10], the rat gut model usually requires the use of many live animals, which may limit its routine use in early discovery projects. Avdeef and Tam [11] have developed a biophysical model for predicting fraction of drug absorb in human based on published human jejunal permeability (P_{eff}) that were correlated with measured Caco-2 permeability. In this work, we seek to extend this biophysical model to predict human jejunal permeability, and fraction absorbed in human (Fa) for some of the selected FQs, using measured Caco-2 permeability. It is noted that values of P_{eff} have not been reported for these FQs, and not all of the compounds have reported fraction absorb data in human.

2. Biophysical model

Caco-2 apparent permeability values (P_{app}), were collated from various literature sources, and pre-treated with the pCEL-X program (in-ADME Research, NY, USA), to obtain transcellular permeability values at pH 6.5. In brief, this involved removing the effect of paracellular (para) and aqueous boundary layer (ABL) permeability contributions from the apparent Caco-2 permeability value to obtain the transcellular (trans) permeability value using Eq. (1) [12,13]:

$$\frac{1}{P_{app}} = \frac{1}{P_{ABL}} + \frac{1}{P_{trans} + P_{para'}} \tag{1}$$

The transcellular permeability value is then adjusted to the pH of interest using Eq. (2) [11]:

$$\frac{1}{P_{trans}} = \frac{10^{-pH+pK_{a,1}} + 10^{+pH-pK_{a,2}} + 1}{P_{\pm/0}} \tag{2}$$

where $pK_{a,1}$ and $pK_{a,2}$ are the ionization constants of the FQ, and $P_{\pm/0}$ is the intrinsic permeability of the zwitterion/ampholyte (net zero charge) species.

For the modeling of human jejunal permeability (P_{eff}), Eq. (3) was used [11]:

$$\frac{1}{P_{eff}} = \left(\frac{1}{P_{eff}^{ABL}} + \frac{1}{P_{eff}^{trans} + P_{eff}^{para}} \right) = \frac{1}{k_{VF}} \left(\frac{h_{ABL}}{D_{aq}} + \frac{1}{P_c^{6.5} + P_{para}} \right) \tag{3}$$

Table 1

Biophysical human P_{eff} model parameters used in this study

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

where h_{ABL} represents ABL thickness in the human *in situ* jejunal perfusion experiment. D_{aq} (cm^{2}s^{-1}) represents the aqueous diffusivity of the drug, which was estimated at 37°C using an empirical function of the molecular weight (MW): $D_{aq} = 0.991\times10^{-4}$ MW$^{-0.453}$. The symbol P_{para} represents the paracellular permeability, and k_{VF} represents the jejunal surface area expansion factor [11].

As a minor extension to the paracellular model of Adson et al. [14], 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 $\varepsilon/\delta2$ low-capacity, size- and charge-independent pathways. This dual-pore population paracellular equation can be written as:

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

(4)

where $E(\Delta\varphi)$ represents function due to potential drop across the cell junction (with values of 1.01-1.22 for the selected FQs), F is the Renkin pore sieving equation (with values of 0.07-0.11 for the selected FQs), a function of ratio of r_{HYD}, the Sutherland-Stokes-Einstein radius and R, the junctional pore radius [11]. The parameters used in this study have already been reported in reference 11 and are summarized in Table 1. Details of the derivation and parameter optimization are not repeated here. Readers are directed to the relevant literature [11] for the methodological details.

With the P_{eff} values predicted using Eq. (3), the following standard exponential function was used to model the %F_a [15]:

$$\%F_a = \left(1 - e^{-(\frac{2}{R'}P_{eff}t_{res})}\right)100\%$$

(5)

where R' represents the radius of small intestine, and is set to 2 cm. The symbol t_{res} represents the small intestine residence time, and is set to 2.8 h. This value was derived from the best fit between the literature and calculated %F_a values, which is in line with the average small intestine residence time of 1-3 h in human [16].

3. Results and discussion

As shown in Table 2, our modeling results revealed that most of the fluoroquinolones are ABL limited in their permeation. Except norfloxacin and ciprofloxacin, where the paracellular component

Table 2

Predicted human jejunal permeability (P_{eff}) and fraction absorb (%F_a) in human of selected fluoroquinolones

Compound	$P_C^{6.5\,a}$ (10^{-6} cm s^{-1})	P_{eff} (10^{-4} cm s^{-1})	%ABL	%para	%trans	%F_a (calc)	%$F_a^{\,b}$ (lit)
Norfloxacin	1.9 [17]	0.7	15	23	62	51	35 (30-40) [26]
Ciprofloxacin	4.2 [17]	1.2	25	10	65	76	81 (70-85) [26]
Enoxacin	41 [22]	3.6	74	0	26	99	100 [20]
Lomefloxacin	24 [21]	3.0	64	1	35	97	97 (95-98) [26]
Ofloxacin	23 [17]	3.0	63	1	36	97	100 [18]
Pefloxacin	232 [19]	4.5	100	0	0	100	100 (80-110) [23]
Sparfloxacin	54 [17]	3.6	80	0	19	99	100 [26]
Gatifloxacin	7.3 [21]	1.7	37	4	59	87	100 [26]
Moxifloxacin	353 [21]	4.2	96	0	4	99	NA^c
Trovafloxacin	119 [25]	3.9	90	0	10	99	96 (65-122) [24]

Note: ^{a}Transcellular permeability corrected for the paracellular and aqueous boundary layer effects, and pH adjusted to 6.5 (see text). The references quoted referred to the original Caco-2 permeability data.
^{b}Fraction absorbed in human (corrected for first pass metabolism as appropriate). Ranges are quoted within the parentheses as absolute bioavailability where multiple values are available. The references quoted referred to the original bioavailability data.
^{c}Not available

are 23% and 10% respectively, most of the compounds do not appear to show significant paracellular transport. The three least-absorbed were norfloxacin, ciprofloxacin and gatifloxicin. Norfloxacin was predicted to have the highest paracellular route = 23% with ABL-limited transcelluar = 15% and transcellular = 62%. Ciprofloxacin have the highest transcellular = 65% with ABL-limited transcelluar = 25% and paracellular route = 10%.

The calculated %F_a for norfloxacin = 51% (lit. 35%) and for ciprofloxacin = 76% (lit. 81%).   Most of the FQs (e.g. enoxacin, lomefloxacin, ofloxacin, pefloxacin, sparfloxacin) showed calculated %F_a > 90%, and are expected to be well-absorbed. It can be seen that the calculated %F_a values are in good agreement with literature data, where available (see Table 2).

As shown in Table 2, the predicted P_{eff} (in 10^{-4} cm·s^{-1} units) ranged from 0.7 (norfloxacin) to 4.5 (pefloxacin). It has been reported that drugs with a P_{eff} value > 1.5 will be completely absorbed no matter which transport mechanism(s) are utilized [15]. Our predictions suggest that only norfloxacin and ciprofloxacin with P_{eff} value < 1.5, which are consistent with literature data on the fraction absorbed of these two drugs indicating incomplete absorption in human. Table 2 shows that the P_{eff} values for other drugs are greater than 1.5, suggesting completed absorption. These are in excellent agreement with the reported fraction absorbed data.

4. Conclusion

We have shown that the estimates of the human jejunal permeability (P_{eff}) can be predicted by the biophysical model, which was developed by Avdeef and Tam [11], starting from measured Caco-2 permeability values at pH 6.5. It has been shown that the fraction absorbed values in human can be predicted using these calculated P_{eff} values. It is envisaged that this model could be a useful predictive tool to estimate P_{eff} and fraction absorbed in human in the development of new fluoroquinolones.

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