

THREE COMPARTMENT MODEL TO DESCRIBE ATIPICAL PERMEABILITY PROFILES IN CACO-2 CELLS

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Introduction

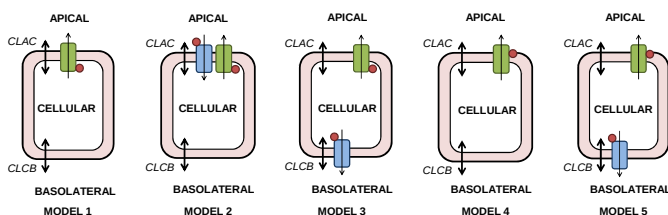
Successful development of new oral drugs must include testing of their absorption. Fexofenadine HCl (FEX), a second generation non-sedating histamine H1 receptor antagonist, is an active metabolite of terfenadine¹. Oral formulations at a dose of 60-120 mg/day are available for FEX. Permeability of FEX was determined in an in vitro three compartment model² at different donor concentrations, and the effect of sodium dodecyl sulfate (SDS) at two concentration levels (10 and 50 µM) on FEX permeability was evaluated.

Objectives

- ✓ To evaluate the ability of the Caco-2 system to detect the secretion process.
- ✓ To apply a three compartment model analysis to the Caco-2 data in order to elucidate the influence of the carrier location and binding site on the transport process.
- ✓ To elucidate the mechanism of surfactant effect in the transport process.
- ✓ To show the utility of kinetic modelling to investigate membrane transport.
- ✓ To compare different approaches for permeability estimation from the raw data i.e. linear versus non linear approximation, sink versus non sink assumptions and constant versus time dependent permeability.

Materials and Methods

The in vitro transport studies were developed in Caco-2 cell in apical-to-basolateral (AB) and basolateral-to-apical (BA) direction. Permeability values were estimated by non-linear regression of concentrations in the receptor chamber, and remaining concentrations in donor chamber versus time. The basic kinetic model comprises three compartments, donor, cell and acceptor chamber. The fitting procedures were performed using NONMEM 7.2 software with the first order conditional estimation method (FOCE) for objective function estimation and ADVAN9 subroutine. Several kinetic models were fitted to the data, functional and mechanistic models, selecting the best model with lowest objective function value. Some of them, are represented below. The inter-individual and residual variabilities of the kinetic parameters were described with the use of exponential models. Goodness of fit plots and visual predictive check were generated in the aim of confirm the final model selected.



$$CLAC_i = PAC_i \cdot S$$

$$CLAC = (CLAC_i - CLACF) \cdot \left(1 - \frac{T^n}{T_c^n + T^n}\right) + CLACF$$

$$CLCB = CLAC \cdot FACT$$

$$\frac{dQ_A}{dt} = CLAC \cdot (C_C - C_A) + \frac{V_{max} \cdot C_C}{(K_M + C_C)}$$

$$\frac{dQ_C}{dt} = -CLAC \cdot (C_C - C_A) + CLCB \cdot (C_B - C_C) - \frac{V_{max} \cdot C_C}{(K_M + C_C)}$$

$$\frac{dQ_B}{dt} = -CLCB \cdot (C_B - C_C)$$

Inter-individual variance

$$PAC_i = PAC \cdot e^{\eta_{PAC}} \quad \eta_{PAC} \in N(0, \sigma_{PAC}^2)$$

Intra-individual variance

$$C_{ij} = C_i \cdot e^{\epsilon} \quad \epsilon \in N(0, \sigma^2)$$

The analysis of A-B and B-A permeabilities suggested the existence of an efflux mechanism. The best model fit corresponded to a kinetic model with passive mechanism and an efflux carrier located at the apical side of the enterocyte (Model 1). The OFV obtained was -89.98 and the number of parameters was 22. The presence of high concentration of surfactant (50 mM) affects to the passive diffusion of FEX differently for each direction of transport.

Goodness of fit plots were generated to confirm the capacity of the model to describe experimental data and, as it can be observed in Figure 2, Figure 3 and Figure 4, there is no bias for observed concentrations versus populations predictions.

Visual predictive check (Figure 5) of the final population model revealed excellent predictive performance for FEX with/without SDS and variability of the observed concentrations were well captured

PARAMETER	VALUE
$P_{diff\ AC} \text{ FEX-SDS } 10 \mu\text{M} (\text{cm} \cdot \text{s}^{-1})$	$1.39 \cdot 10^{-6}$
$P_{diff\ AC} \text{ SDS } 50 \mu\text{M} \text{ AB} (\text{cm} \cdot \text{s}^{-1})$	$50.2 \cdot 10^{-6}$
$P_{diff\ AC} \text{ SDS } 50 \mu\text{M} \text{ BA} (\text{cm} \cdot \text{s}^{-1})$	$7.27 \cdot 10^{-6}$
$P_{diff\ INITIAL} \text{ AB}$	$14.8 \cdot 10^{-6}$
$P_{diff\ INITIAL} \text{ BA}$	$9.25 \cdot 10^{-6}$
$P_{diff\ INITIAL} \text{ SDS}$	$228 \cdot 10^{-6}$
$P_{diff\ INITIAL} \text{ SDS}$	$47.1 \cdot 10^{-6}$
FACT	3.14 FIX
Vc (mL)	$1.67 \cdot 10^{-5}$
V_{max} (ng·seg ⁻¹)	1550
KM (µM)	500 µM
TLAG AB (sec)	870
TLAG BA (sec)	847
TLAG SDS	50 µM+50
TLAG SDS	1530
TLAG SDS	100 µM
TLAG SDS	332
TLAG SDS	50 µM
TLAG SDS	885
TLAG SDS	50 µM+10
TLAG SDS	562
TLAG SDS	50 µM+50
TLAG SDS	959
V_{max} (ng·seg ⁻¹)	$4.45 \cdot 10^{-3}$
KM (µM)	23.8
Tc (sec)	177
IVV $P_{diff\ AC}$	$2.2 \cdot 10^{-3}$
σ	0.132

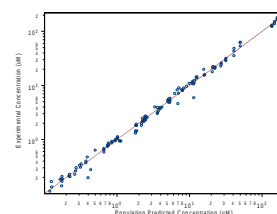


Figure 2. Experimental versus population predicted concentrations (µM)

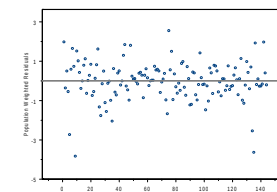


Figure 3. Population weighted residuals versus experimental concentrations (µM)

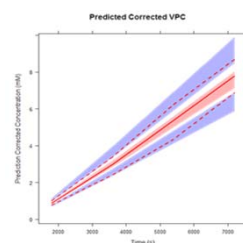


Figure 5. Predicted corrected visual predictive check. Lines are referred to percentiles 5, 50 and 95 and shaded regions based on 99% confidence intervals estimated from simulated data.

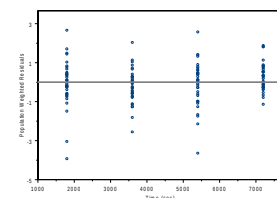


Figure 4. Population weighted residuals versus time (sec)

Results and Discussion

Three different profiles, described by Tavelin³, were observed plotting concentrations versus time (Figure 1). Tlag parameters in cases described by profile 1 were used. In cases similar to profile 3, permeability as a function of time were used to account for the intercept of the concentrations permeated versus time that was different from 0.

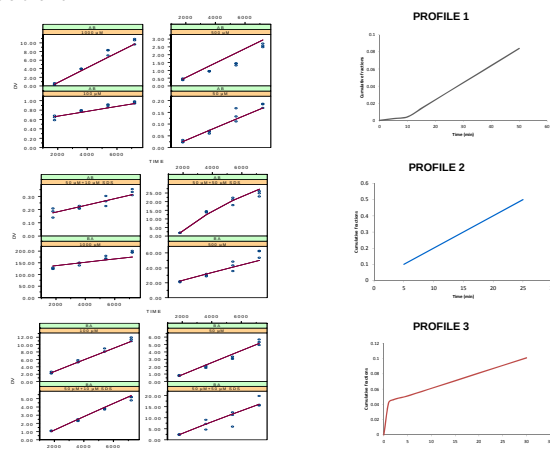


Figure 1. Experimental and population-predicted concentrations versus time in receptor chamber classified by experiment direction (AB-BA) and group of dose.

Conclusions

Fexofenadine has a low intestinal permeability in vitro in Caco-2 cells and the transport of the drug is concentration-dependent due to the involvement of Pgp. Nevertheless, the drug also permeates through the paracellular pathway because it has been shown that SDS is able to increase FEX permeability by relaxing the tight junctions and thus decreasing TEER values, while keeping the monolayer integrity at the concentrations assayed. The three compartment modeling based on differential equations improved the basic approach for parameter estimation (fitting permeabilities versus donor concentration) as it allow to characterize apical and basolateral membrane permeabilities, beside having the advantage of its broader applicability i.e with non sink conditions and time dependent permeability.

References

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