The role of paracellular and carrier-mediated pathways in the nonlinear absorption of BCS Class III substrates for influx and efflux transporters.

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Abstract:

Mechanistic oral absorption simulation of nonlinear dose dependence for BCS Class III transporter substrates is an important objective in preclinical development of NCEs as well as in generic formulation and bioequivalence. This study demonstrates the application of the mechanistic Advanced Compartmental Absorption and Transit (ACAT™) model to simulate the nonlinear dose dependence of gabapentin and celiprolol absorption. Modeling of transcellular, paracellular, and carrier-mediated absorption across a range of doses illustrates the significance of the paracellular pathway for these types of substrates.

We used *in vitro* data for K_m and fitted the *in vivo* V_{max} value for interaction of gabapentin with Organic Cation Transporter 1 (OCTN1) and L-Type Amino acid Transporter 2 (LAT2, SLC7A8) [1] [2] and for the interaction of celiprolol with Organic Anion Transporter 2B1 (OATP2B1) and P-glycoprotein (P-gp) [3] [4]. A mechanistic oral absorption PBPK model that describes the pharmacokinetics of gabapentin and celiprolol in healthy humans was developed using GastroPlus™ (Simulations Plus, Inc.). Physicochemical and biopharmaceutical parameters required for the mechanistic dissolution, absorption, and pharmacokinetic distribution were obtained from the literature or from ADMET Predictor™ (Simulations Plus, Inc.). Tissue:plasma partition coefficients (Kps) were calculated using the Lukacova approach with an experimental value for fraction unbound in plasma.

For gabapentin at a dose of 400 mg, the simulation results show approximately 70% absorbed (Fa) with 20% from the paracellular pathway and 50% from the carrier-mediated pathway (CMP). When the dose is increased to 1600 mg, Fa = 40% with 20% from the paracellular pathway and 20% from the carrier-mediated pathway.

When celiprolol is administered using microdosing (at 37.5 µg) the passive transcellular absorption is directly offset by the influence of P-gp and all of the productive absorption (~2%) occurs via the paracellular pathway. At therapeutic doses (100 mg), celiprolol percent absorbed is 53% with the transcellular and the carrier-mediated pathways each contributing approximately 25% with the paracellular pathway again contributing about 2%.

Absorption of BCS Class III compounds that are substrates for influx and efflux transporters can be complex and requires the accurate calculation of the paracellular contribution to absorption in order to accurately simulate the nonlinear dose dependence. For gabapentin concentrations below the value of the *in vitro* Km, carrier-mediated transport is dominant. However, when the OCTN1 and LAT2 transporters are saturated, the paracellular pathway becomes equivalent to the transporter pathway. For celiprolol, a substrate for influx and efflux transporters, microdosing results in concentrations below the Km for P-gp, resulting in low absorption due only to the passive transcellular component. At therapeutic doses, celiprolol saturates P-gp and OATP2B1 carrier-mediated absorption dominates.

Biopharmaceutical and Clinical Data

The study was conducted using literature data for plasma concentration vs. time for gabapentin [5] and celiprolol [4].

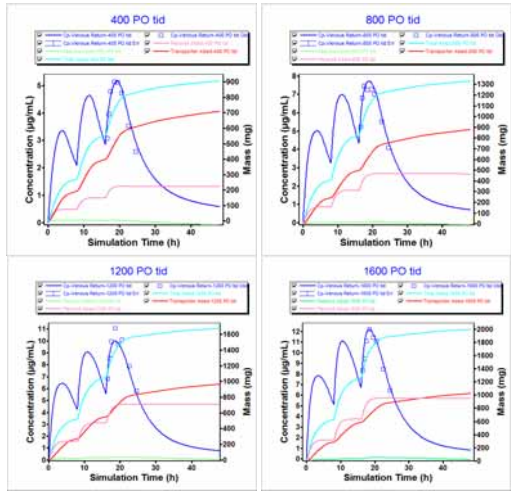
Property	Gabapentin	Celiprolol
Log D(7.4)	-1.1 [2]	0.13 [8]
Passive Permeability	Paracellular only: 0.29 x 10^{-4} cm/s [13]	Paracell. = 0.02 x 10^{-4} cm/s [13] Transcell. = 0.1 x 10^{-4} cm/s fitted
Solubility	100 mg/mL @ pH 7.2	176 mg/mL [8]
Km	OCTN1 = 0.58 mM [6] LAT2 = ~5.0 mM [1]	OATP2B1 = 20.5 µM [9] P-gp = 0.1 µM [fitted]
Vmax	Fitted to *in vivo* data. OCTN1 = 5.8 pmol/min/mg Prot. LAT2 = 1200 pmol/min/mg Prot.	Fitted to *in vivo* data. OATP2B1 = 47 nmol/min/mg Prot. P-gp = 1.9 nmol/min/mg Prot.

Methods

1. Tissue/plasma partition coefficients (Kps) for perfusion-limited tissues were calculated using the Lukacova method based on tissue composition and *in vitro* and *in silico* physicochemical properties (ADMET Predictor™, Simulations Plus, Lancaster, CA). Kps for permeability-limited tissues were calculated based on the ratio of fup/fut.
2. **Clearance** for **gabapentin** was simulated using only fup*GFR in kidney. Clearance for **celiprolol** included fup*GFR and a permeability–limited liver with passive permeability surface area product (PStc) of 100 mL/s for both basolateral and apical membranes.
3. The discontinuities and double peaks seen in the celiprolol Cp vs. time profile were modeled by assuming two phases of gastric emptying.

Results

Gabapentin:

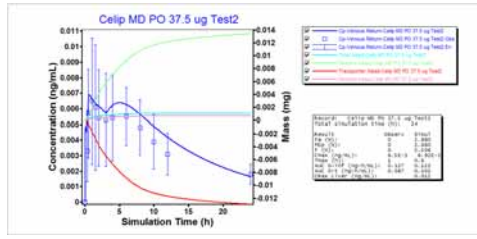


Simulation of the nonlinear dose dependence for gabapentin using tid dosing. Here we assumed OCTN1 influx on the apical membrane and LAT2 efflux on the basolateral membrane of the enterocyte.

Dose	Bioavailability
400 mg	71%
800 mg	53%
1200 mg	45%
1600 mg	40%

The fraction of dose absorbed (Fa) by the transcellular passive mechanism is not significant. The mass absorbed from the paracellular route increases linearly with dose and contributes ~20%. The mass absorbed from the carrier-mediated routes decreases from 58% at 400 mg to 21% at the 1600 mg dose due to saturation.

Celiprolol: microdose

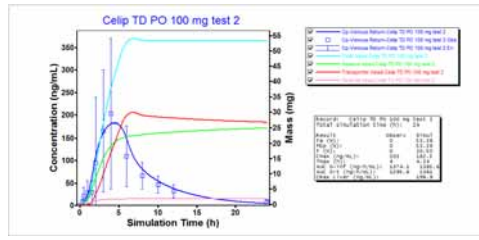


Simulation of the nonlinear dose dependence for celiprolol. Here we assumed OATP2B1 influx and P-gp efflux on the apical membrane of the enterocyte.

Dose	Bioavailability
37.5 µg	2.5%
100 mg	30.5%

Under microdosing conditions, the fraction of dose absorbed (Fa) by the transcellular passive mechanism was completely secreted back into the lumen of the intestine by P-gp [4] and the mass absorbed from the paracellular route (2%) accounted for all the absorption. The mass absorbed from OATP2B1 influx and the passive transcellular route were completely eliminated by P-gp when intraenterocyte concentrations were below the Km for P-gp. Under therapeutic dosing conditions, the passive transcellular route and OATP2B1 influx were approximately equal (25% each) and were able to overcome the P-gp efflux influence. The model included some first-pass extraction due to biliary excretion resulting in 30% bioavailability.

Celiprolol: therapeutic dose



Conclusions:

We have demonstrated the utilization of ACAT™ model simulations to accurately explain the observed nonlinear dose dependence for gabapentin and celiprolol. As we have demonstrated previously [10] the simulations can be parameterized with *in vitro* data for Km of the influx carrier-mediated processes. The Km for efflux of celiprolol by P-gp was much lower than the Km for influx by OATP2B1 resulting in a strong efflux influence under microdosing conditions. Optimization of the Km for P-gp required fitting to the *in vivo* data as we have demonstrated previously [11, 12].

The inclusion of a paracellular pathway in these simulations was critical to explain all of the observed data. Gabapentin is a small zwitterionic molecule and consequently would be expected to be partially absorbed through the paracellular route. This pathway bypasses both influx and efflux transporters and provided a linear percentage absorption of the administered dose in our simulations. Saturation of the influx transporters results in the paracellular pathway becoming equivalent to the carrier-mediated pathways. Celiprolol is larger than gabapentin and has a much smaller contribution of the paracellular pathway. However, according to our simulations, under microdosing conditions, the paracellular pathway provides the only mass absorbed.

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13. Paracellular permeability for gabapentin and celiprolol was calculated by GastroPlus™ using spherical and hydrodynamically equivalent radii calculated by ADMET Predictor™.

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