Investigating the mechanism of acamprosate intestinal absorption by the use of *in vitro* transporter studies in combination with modelling and simulations

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Introduction

Acamprosate is used to treat alcoholism, and modulates neural transmission in the brain. It structurally similar to neurotransmitters. Acamprosate is a small (MW = 181.2), hydrophilic (Log P = -3.57) sulfonic acid (pKa = 1.8) compound. Acamprosate has negligible metabolism and is mainly eliminated renally, which indicates that its poor oral bioavailability (11%) is likely due to poor intestinal absorption.

Aim

The aim of this study is to investigate the intestinal absorption of acamprosate using *in vitro* transporter studies in combination with modelling and simulation techniques.

Methods

Mechanistic model to predict $P_{eff,\ man,\ passive}$

The effective, passive intestinal segmental permeability ($P_{eff,\ man,\ passive}$) is a function of the unstirred boundary layer (UBL) permeability (P_{UBL}), the diffusion-driven transcellular- (P_{trans}) and paracellular permeabilities (P_{para}) and an intestinal segment scalar (k_{GI}). $P_{eff,\ man,\ passive}$ was estimated using a newly-developed, mechanistic, physiologically-based absorption model in MatLab (Equations below[1,2], Table 1, Figures 1 and 2).

$$P_{eff,man,passive} = \left(P_{trans} + P_{para}\right) \times k_{GI} \times \frac{P_{UBL}}{(P_{trans}+P_{para})+P_{UBL}}$$

$$P_{para} = \frac{\varepsilon}{\delta} \times D \times F\left(\frac{r}{R}\right) \times (f_0 + f_z \times 0.24)$$

$$P_{trans} = 2.36 \times 10^{-6} \times P_{o:w}^{\ 1.1}$$

$$k_{GI} = VE \times FE \times Acc$$

$$P_{UBL} = D/h_{UBL}$$

Table 1. Parameters for mechanistic model

Compound parameters	
Accessibility surface scalar (Acc)	1
(Spherical) molecular radius (r) [Å]	3.6
Diffusion coefficient (D) [x 10^{-6} cm^2/s]	9.39
fraction of neutral species (f_0)	(~)0
fraction of negatively charged species (f_z)	1
Octanol-water partition coefficient ($P_{o:w}$)	0.000269

System parameters	
Paracellular pore radius (R) [Å]	
Duodenum/Jejunum	8.8
Ileum	3.8
Colon	2.3
Villi Expansion (VE)	
Duodenum/Jejunum	10
Ileum	10
Colon	1
Fold Expansion (FE)	
Duodenum/Jejunum	3
Ileum	1
Colon	1
Porosity/Pore-length (ε/δ) [cm^{-1}]	0.466
UBL height (h_{UBL}) [μm]	30

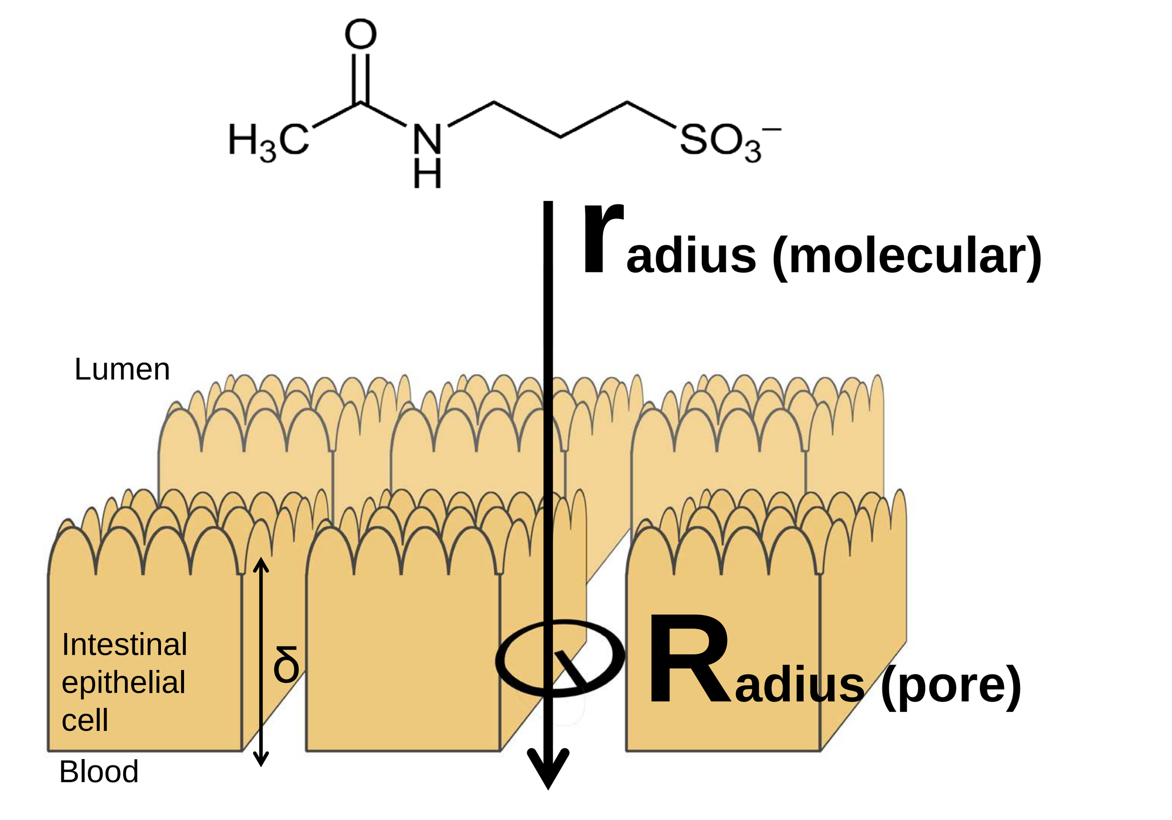


Figure 1. Schematic representation of a selection of mechanistic model parameters used to calculate P_{para}.

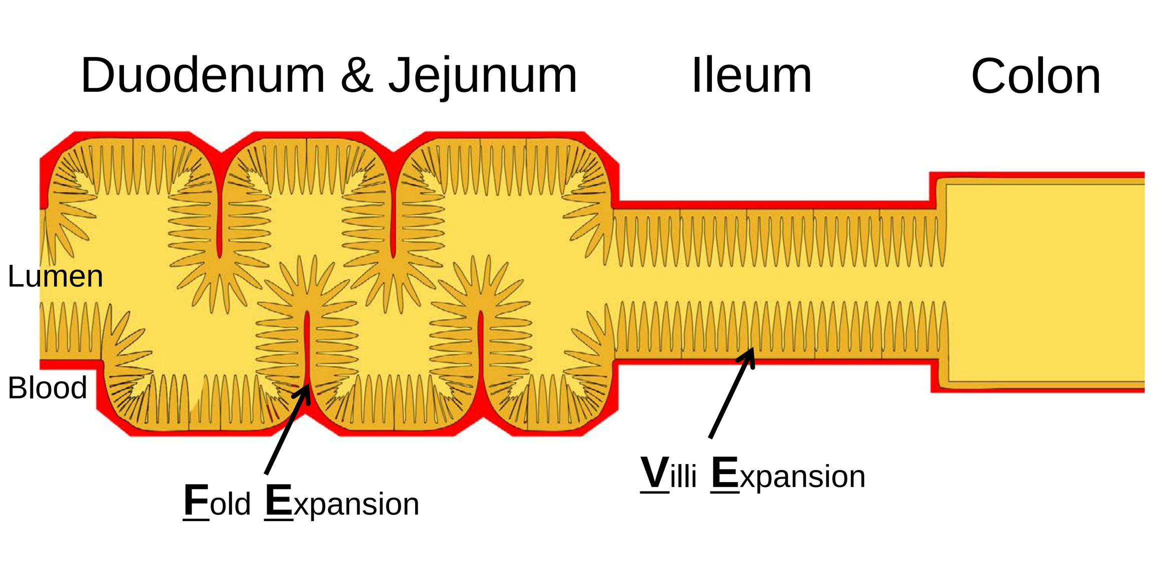


Figure 2. Schematic representation of intestinal morphology used to scale the area available for permeation.

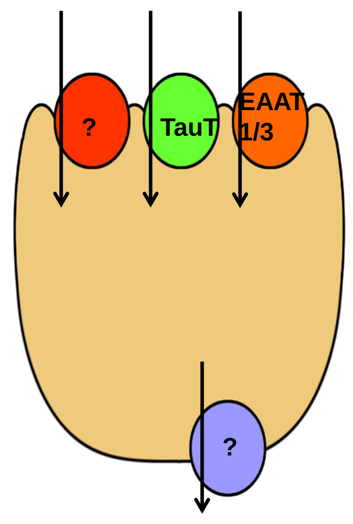
Methods, continued

PBPK model

Physiologically-based pharmacokinetic (PBPK) modelling of acamprosate, using the estimated $P_{eff,\ man,\ passive}$, was performed by the "Advanced Dissolution, Absorption and Metabolism" (ADAM) model within the Simcyp Population-based Simulator (V12-R2). The ADAM model incorporates the specific population variability of gastric emptying time, intestinal transit-time, length, pH *etc.*[3] As performance verification of the full PBPK-model, simulated area under the plasma concentration-time profile (AUC) and oral fraction absorbed (fa), following single intravenous or oral acamprosate doses over a range of 666-2310 mg, were compared to reported data for healthy volunteers.

In vitro transporter studies

Acamprosate inhibition of radiolabelled substrate influx or efflux by nine selected transporters was investigated *in vitro* using Caco-2 cells from "Deutsche Sammlung von Mikroorganismen und Zellkulturen" (DSMZ) (Figure 3). Passage numbers 2-11 were used and uptake studies were conducted on day 11 or 21 after seeding if cultured on bottom of wells or on filters, respectively. In the cases when acamprosate inhibited substrate influx, *i.e.* for [^{3}H]-taurine and [^{3}H]-glutamate via TauT and EAAT1/3, respectively, IC_{50} was determined using substrate concentrations of 37.6 and 9.8 nM, respectively.



Investigated apical influx transporters *(gene)* (substrate): Peptide transporter 1 (PEPT1/*SLC15A1*) ([^{14}C]-Gly-Sar), Taurine transporter (TAUT/*SLC6A6*) ([^{3}H]-taurine), H^+/Amino acid transporter 1 (PAT1/*SLC36A1*) ([^{3}H]-proline), Excitatory amino-acid transporters (EAAT1/3/*SLCA3/1*) ([^{3}H]-glutamat), Glycoprotein-associated amino acid transporter/Neutral and basic amino acid transport protein ($B^{0,+}$AT/rBAT/*SLC7A9/SLC3A1*) ([^{3}H]-lysine), Organic anion transporter polypeptide 2B1 (OATP2B1/*SLCO2B1*) (^{3}H-Estrone-3-sulfate), Apical sodium dependent bile acid transporter (ASBT/*SLC10A2*) ([^{3}H]-taurocholic acid)

Investigated basolateral, bi-directional transporter (*gene*) (substrate): Organic solute transporter α/β (OST α/β/*SLC51A/B*) *(3H-Estrone-3-sulfate)*

Figure 3. Overview of investigated transporters by acamprosate inhibition of substrate influx/efflux.

Results

P_{para} determines $P_{eff,\ man,\ passive}$

- P_{UBL} was calculated to 31.3•10^{-4} cm/s, and does not limit P_{para} and P_{trans}.
- It is mainly P_{para} that contributes to overall acamprosate $P_{eff,\ man,\ passive}$ (Table 2).
- P_{para} is to a large extent influenced by the molecular- to pore-radius ratio (r/R) (Figure 4). As acamprosate r/R increases, P_{para} decreases almost exponentially.

Table 2. Estimates of passive permeabilities used in simulation of acamprosate absorption, and corresponding mean fraction absorbed (fa)

Intestinal segment	r/R	P_{para} (10^{-6} cm/s)	P_{trans} (10^{-6} cm/s)	k_{GI}	$P_{eff,\ man,\ passive}$ (10^{-4} cm/s)	fa
Duodenum/ Jejunum	0.41	0.0979	0.0003	30	0.0293	0.0055
Ileum	0.95	0.0001	0.0003	10	<0.0001	<0.0001
Colon	>1	0	0.0003	1	<0.0001	<0.0001

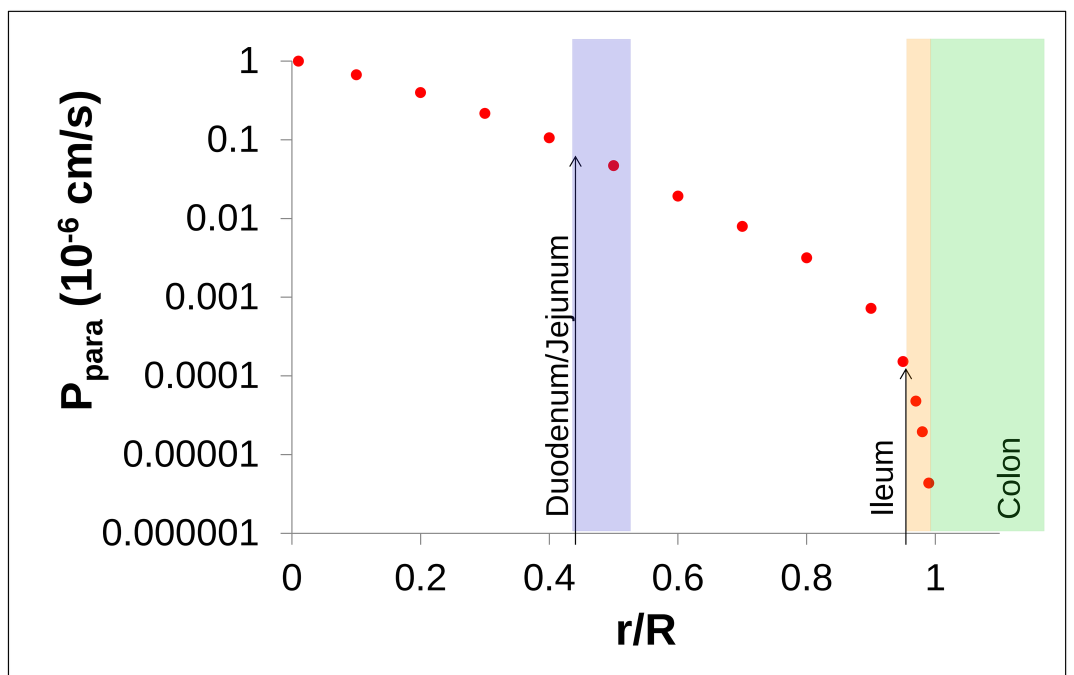


Figure 4. Relation between paracellular permeability (P_{para}) and the molecular- to pore-radius ratio (r/R). The molecular radius for acamprosate was calculated as 3.6 Å.

Results, continued

P_{para} underestimates acamprosate oral exposure

The simulation of disposition and clearance of acamprosate is, for our purpose, acceptably predicted (Figure 5, top). Hence, it is the estimation of acamprosate oral absorption -and not disposition or clearance- that mainly discriminates between the simulated and observed plasma-concentration-time profiles after oral administration of acamprosate.

The simulation of acamprosate plasma concentration-profiles AUC after oral administration, using segmental $P_{eff,\ man,\ passive}$ input (which almost exclusively is determined by P_{para} (Table 2.), under-predicts acamprosates AUC (Figure 5, bottom). Adding, by fitting, net absorptive carrier-mediated permeation ($P_{carrier}$) to $P_{eff,\ man,\ passive}$, improves the prediction of acamprosates plasma concentration-time profile.

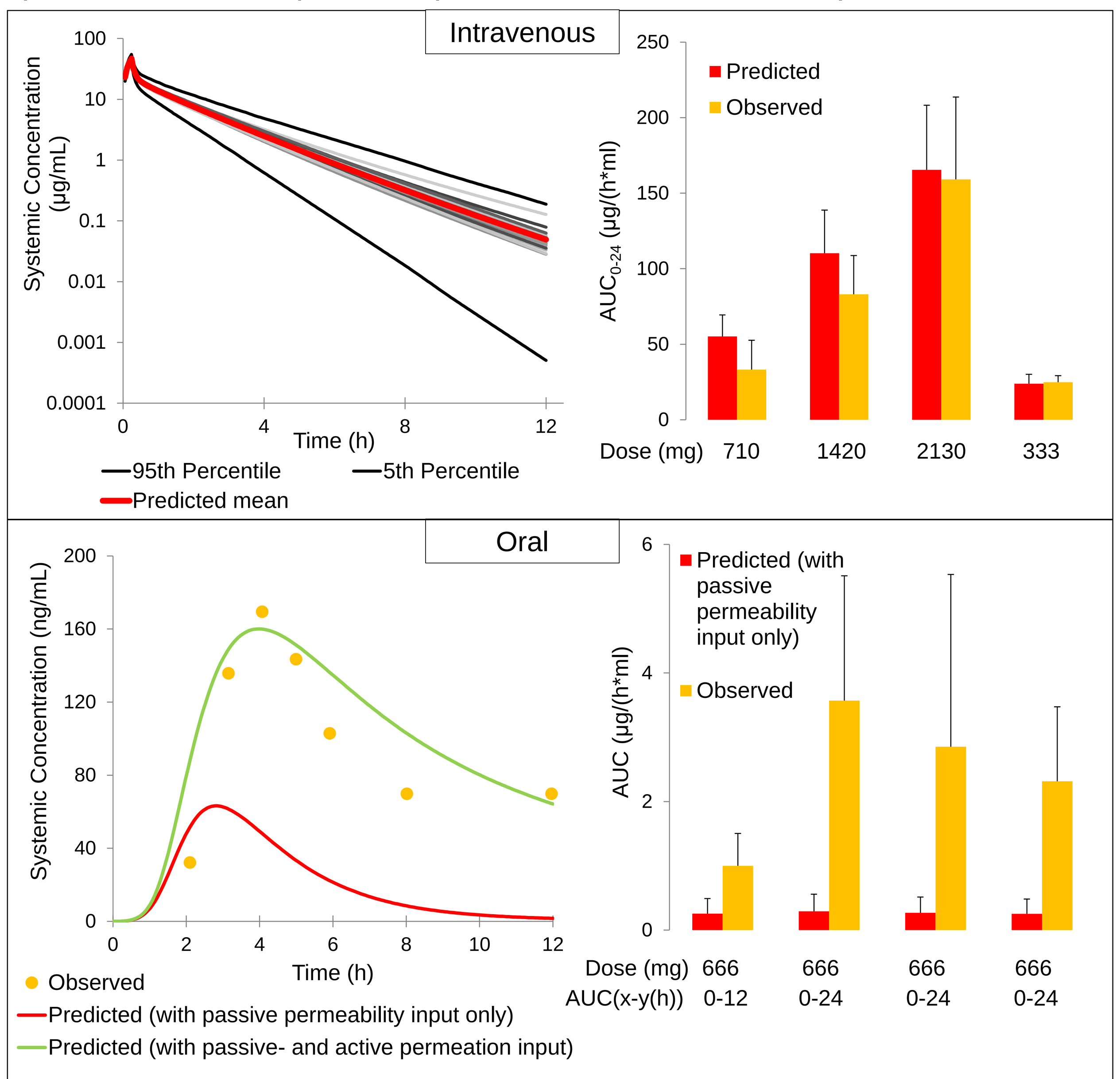


Figure 5. Acamprosate PBPK model simulation of plasma concentration-time profiles and AUC compared to clinical studies. All studies are single-dose studies. The concentration-time profiles are given after a 666 mg dose.

Acamprosate inhibits carrier-mediated taurine and glutamate uptake

Acamprosate did not inhibit substrate uptake by the intestinal transporters shown in Figure 3, except for [^{3}H]-taurine uptake via TAUT and [^{3}H]-glutamate uptake via EAAT1/3, which resulted in the IC_{50} values of 69.1 and 183.3 mM, respectively.

Conclusions

- Acamprosate intestinal absorption in human seems to be partly paracellular and partly (currently unidentified) transporter mediated.
- Acamprosate intestinal absorption in human is negligibly influenced by diffusion-driven transcellular- and unstirred boundary layer permeability.
- The investigated transporters (Figure 3) seem not to contribute in great extent to acamprosate carrier-mediated permeability.
- Further *in vitro* transporter studies are required to identify which other transporters may be involved in acamprosate absorption. Corresponding *in vivo* intestinal transporter abundance and activity data are then desirable to allow *in vitro-in vivo* extrapolation for these transporters.

In vitro transport studies in combination with modeling and simulation is a powerful combination of tools to investigate mechanisms of intestinal absorption.

References

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