Physiologically-based Pharmacokinetics (PBPK) Linked to Pharmacodynamics: *In silico* and *in vitro* Parameterization

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Chief Scientist
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Outline

- PBPK background and principles
  - Pioneers who have influenced our thinking
  - Why PBPK compared to compartmental?
  - Perfusion limited vs. Permeability limited
  - Equations for transport and clearance
- *In silico* generation of organ physiology (PEAR)
- *In silico* calculation of tissue:plasma partition coefficients (and options)
- Examples

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Pioneers who influenced our thinking

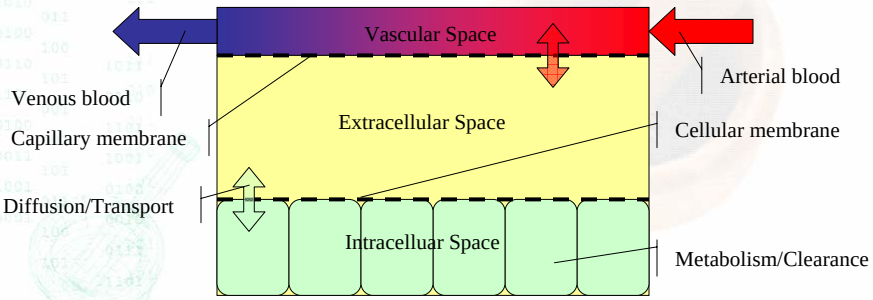
- **Teorell** (1937)
- **Rowland**: Nestorov, Blakey (*iv* barbiturates), Kawai (*iv, po* cyclosporin), Rodgers (*iv, po* β-blockers)
- **Stanski:** Wada (*iv* thiopental)
- **Krishnan**: Poulin (*in silico* Kps), Haddad (*in silico* organ physiology)
- **Price** (*in silico* organ physiology)
- **Brown** (*in vivo* organ physiology)
- **Sugiyama, Holford, Houston**

Why PBPK?

- Physically relevant model
- Amenable to inter-species scaling
- Simulate Cp vs. time from *in vitro* data
- Explore PK as function of physiology
  - Disease states
  - Variability

Tissue Models

- A fairly complex model:



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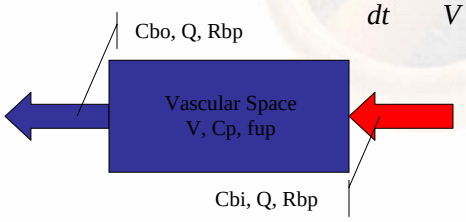
Tissue Models

- Blood Compartment:
  - Well Mixed
  - No clearance
  - Linear binding
  - Rapid RBC penetration

$$\frac{dCbo}{dt} = \frac{Q}{V}(Cbi - Cbo)$$



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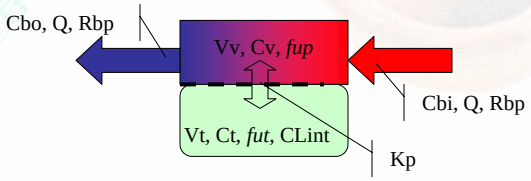
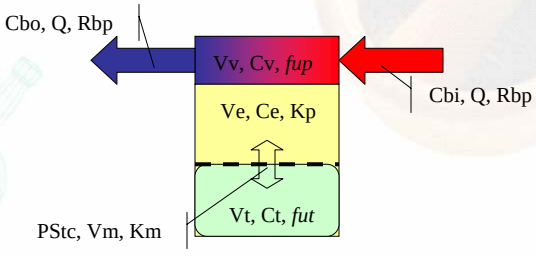
Tissue Models

- Perfusion Limited Tissue:
  - Well Mixed
  - Rapid membrane permeation
  - Same unbound concentration in interstitial and intracellular space
  - Preferential partitioning to tissue (Kp)

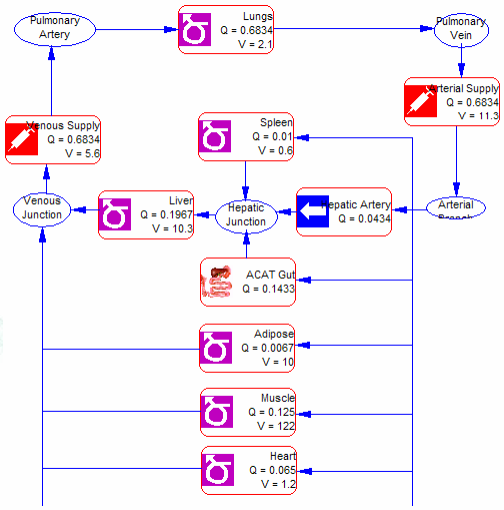


Tissue Models

- Permeability Limited Tissue
  - Slow permeation across cell membranes
  - Unbound concentrations in intracellular and interstitial space are different
  - Only unbound drug permeates or is transported



Global Model



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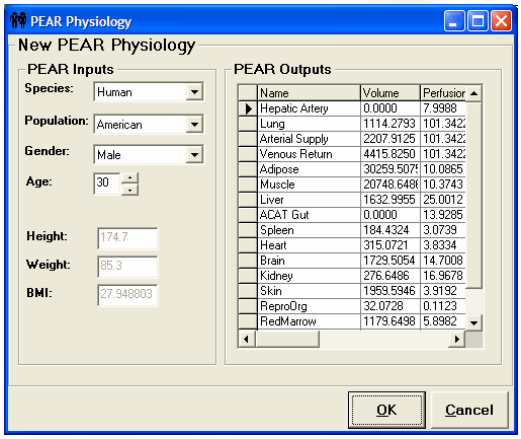
GastroPlus Generates PBPK Parameters for You

PEAR Physiology™ Generator
- American/Western
- Japanese/Asian
- Rat (single physiology)

Automatically generates all tissue parameters for selected ethnic group, gender, and age.

Generates random samples for Virtual Trials



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National Health and Nutrition Examination Survey (NHANES) 2001 – 2002 data

- 11,039 people participated
  - 5331 males, 5708 females
  - 3293 Hispanic
  - 4606 Non-Hispanic White
  - 2681 Non-Hispanic Black
  - 459 other race
- Collected Weight, Height, BMI, and bioelectrical impedance (R = Ohms).

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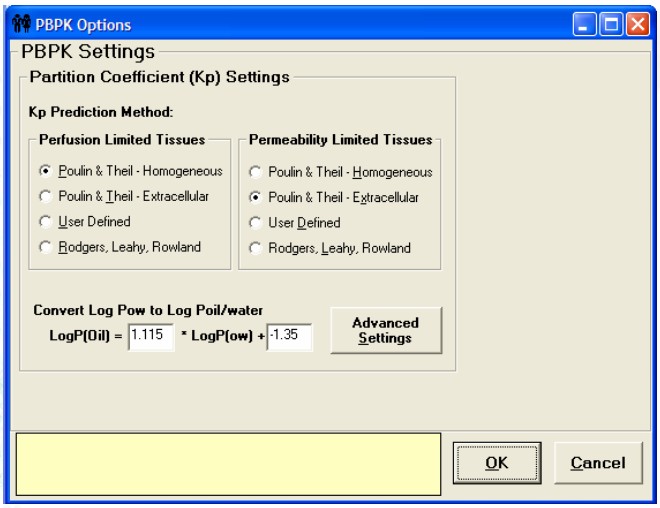
Kp Calculation Options



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(Advanced Settings)

In Silico Tissue Distribution

- Predicting Tissue/Plasma Partition (Kp):
  - Poulin & Thiel

$$Kp = \frac{\left[K \cdot \left(V_{nlt} + 0.3V_{pht}\right)\right] + \left[1 + \left(V_{wt} + 0.7V_{pht}\right)\right]}{\left[K \cdot \left(V_{nlp} + 0.3V_{php}\right)\right] + \left[1 + \left(V_{wp} + 0.7V_{php}\right)\right]} \cdot \frac{fu_p}{fu_t}$$

$$\text{adipose} : K = D^*_{vo:w}$$

$$\text{other} : K = P_{o:w}$$

$$\log P_{vo:w} = 1.115 \log P_{o:w} - 1.35 \quad \text{Leo, Hansch}$$

$$fu_t = \left[\left(1 + \left(1 - fu_p\right)/\ fu_p\right) \cdot RA_{tp}\right]$$

V_{nlt}, V_{pht}, V_{wt} : Volume fraction of neutral lipids, phospholipids, water

RA_{tp} : Albumin ratio tissue : plasma

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GastroPlus Generates PBPK Parameters for You

Rodgers and Rowland Kps:

$$Kp = Kpu * fup$$

Unbound tissue plasma partition coefficient, *Kpu*, is calculated differently for strong bases than for other drugs.

1. **Strong bases and zwitterions with at least one base pKa $\geq$ 7** – takes into consideration the unique interaction of bases with acidic phospolipids (details)

$$Kpu = V_{ewt} + \left(\frac{(1/X_{[D],IW})}{(1/X_{[D],P})} V_{iwt}\right) + \left(\frac{Ka[AP]_T((1/X_{[D],IW}) - 1)}{(1/X_{[D],P})}\right) + \left(\frac{K \cdot V_{nlt} + (0.3K + 0.7)V_{pht}}{(1/X_{[D],P})}\right)$$

2. **Acids, Neutrals, and weak bases** – takes into account binding to lipoproteins (neutral drugs) or tissue albumin (acids and weak bases)

$$Kpu = \frac{(1/X_{[D],IW})V_{iwt}}{(1/X_{[D],P})} + V_{ewt} + \left(\frac{K \cdot V_{nlt} + (0.3K + 0.7)V_{pht}}{(1/X_{[D],P})}\right) + \left[\left(\frac{1}{fup} - 1 - \frac{K \cdot V_{nlp} + (0.3K + 0.7)V_{php}}{(1/X_{[D],P})}\right) \times RA_t\right]$$

$\boldsymbol{X_{[D]}}$ – fraction of neutral drug species in intracellular water (IW, pH=7) and plasma (P, pH=7.4)
$\boldsymbol{K}$ – vegetable oil/water partition coefficient for adipose tissue and 1-octanol/water partition coefficient for remaining tissues
fup-fraction unbound of drug in plasma, ***Ka*** – association constant of base with acidic phospholipids, $\boldsymbol{[AP]_T}$ – tissue concentration of acidic phospholipids
RA_t – tissue/plasma lipoprotein or albumin ratio

Factors Impacting Bioavailability

- **Physiological**
  - pH
  - Transit Time
  - Gastric Emptying
  - GI Dimensions
  - Liver Blood Flow
  - Species
  - Sex
  - Food Effects
- **Biochemical**
  - Plasma Protein Binding
  - Liver Enzymes
  - Gastrointestinal
    - Metabolic Enzymes
    - Efflux proteins
    - Transporters
  - Pharmacogenomics

Drug and Excipient Interactions with all of the above.

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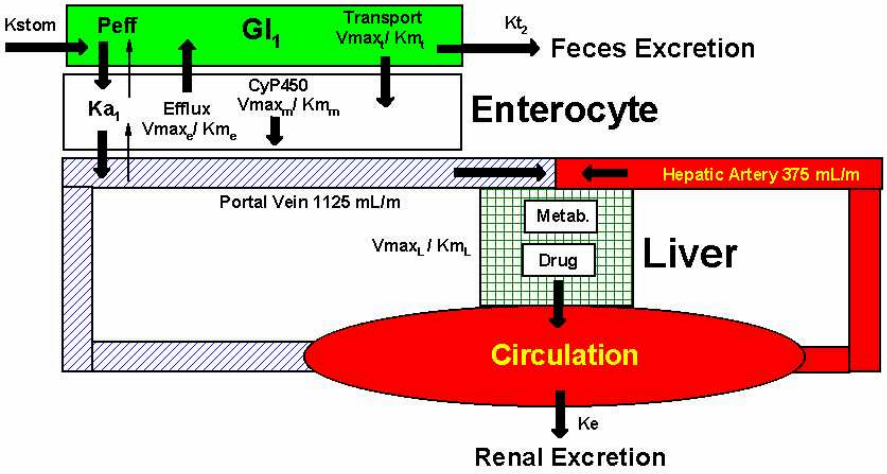
Enterocyte Model for Each Compartment



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Carrier-mediated Transport

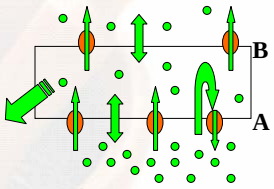
$dM_{ent(i)}/dt$ =

Apical Diffusion Rate

+ Apical Carrier-mediated Transport Rate

- Basolateral Transfer Rate

- Gut Metabolism Rate



Apical Carrier-mediated Transport rate =

$DF_{influx(i)}\ V_{max,influx}\ C_{(i)} / (K_{m,influx} + C_{(i)})$

$- DF_{efflux\ (i)}\ V_{max,efflux}\ C_{u,ent(i)} / (K_{m,efflux} + C_{u,ent(i)})$

DF = distribution factor for transporter amounts *relative to V_{max} measurement environment* (when Vmax in a compartment is the same as Vmax in the measurement environment, then DF = 1.0).

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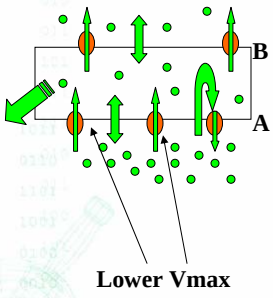
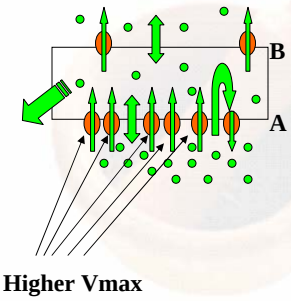
Transporter Distribution Factors





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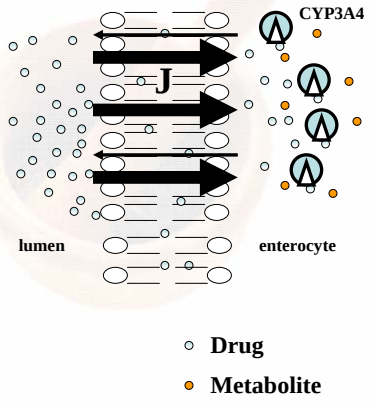
First Pass Metabolism

- Gut wall metabolism can be significant, especially for CYP3A4 and CYP2D6 substrates
- Hepatic first pass is a function of the unbound concentration presented to the liver and hepatic blood flow rate
- Changing absorption location and rate can change *both* gut wall metabolism and hepatic first pass metabolism



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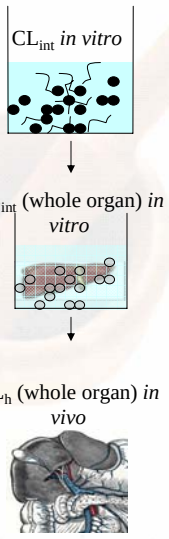
Calculation of hepatic clearance

Houston et al. (1997)

Step 1. *In vitro* incubation of drug with microsomes/hepatocytes/liver slices to obtain enzyme kinetic constants V_{max} and K_m and the *in vitro* intrinsic clearance

Step 2. Scale *in vitro* enzyme kinetic constants to *in vivo* conditions based on species-specific physiological scale factors.

Step 3. Based on a hepatic blood flow model (e.g. Venous equilibrium model [1]), determine *in vivo* hepatic clearance. Rate of drug elimination = $CL_h \times$ Concentration



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Significance of Gut Metabolism and Controlled Release

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Gut Metabolism Scale Factors

Paine MF and Thummel KE, JPET, 1997; 283(3): p. 1552-62.

3A4 (nmol) = 9.7 38.4 22.4

Liver CYP3A = 5489 nmol
Liver Wt. = 1800 g
MicProt = 52.5 mg / g liver
CYP3A4 = 69.7 pmol / mgP

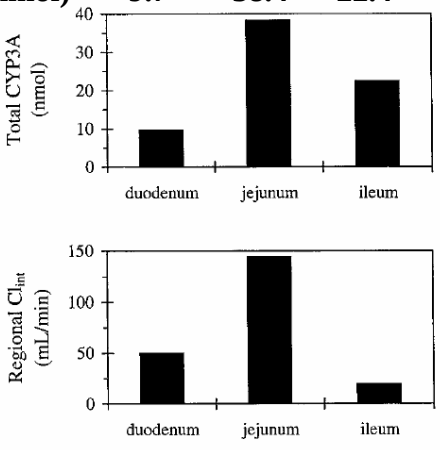

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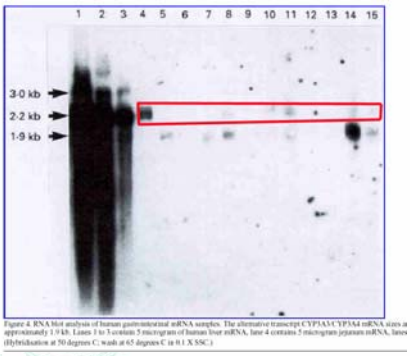
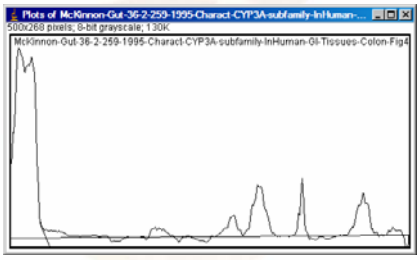
Image J Analysis of Jejunum vs. Colon Metabolism Scale Factors

Liver CYP3A = 5489 nmol
Jejunum CYP3A4 = 38.4 nmol
Colon CYP3A4 = 0.6 - 6.7 nmol





Jejun.....Colon.....
6799 226 409 1171 419 855 110

One Jejunum and 10 colon samples. Samples without Integration numbers were below baseline.

McKinnon, Gut 36(2):259 (1995)

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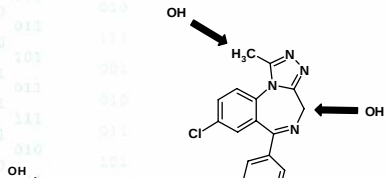
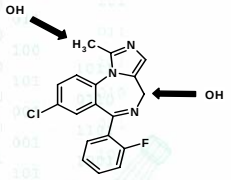
Intestinal and Hepatic CYP 3A4 Metabolism: Midazolam, Alprazolam, and Saquinavir



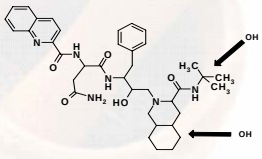
Alprazolam

V_{max} = 2680 pmol/min/mg
K_m = 660 µM
V_{max}/K_m = 4.1
IR Intest. Extract. ~ 1%



Midazolam

V_{max} = 850 pmol/min/mg
K_m = 4 µM
V_{max}/K_m = 212
IR Intest. Extract. = 43%



Saquinavir

V_{max} = 3960 pmol/min/mg
K_m = 0.4 µM
V_{max}/K_m = 9900
IR Intest. Extract. = 64%

Fitzsimmons-DrugMetabDisp-25-2-256-1997-SaquinavirMetabolismIntestine.pdf

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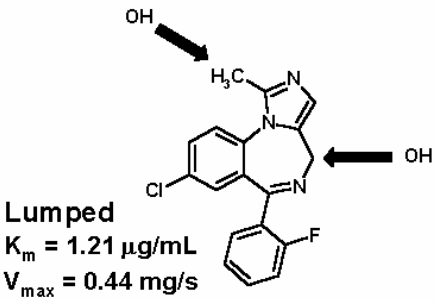
Intestinal and Hepatic CYP 3A4 Metabolism

Midazolam



Lumped
K_m = 1.21 µg/mL
V_{max} = 0.44 mg/s

MWt = 325.8

Log P = 3.37 (Exp.)

pKa =6.15 Base (ADMET Predictor)

Solubility = 8.7 µg/mL @ pH 7.7 (ADMET Predictor)

Peff = 12 x 10^{-4} cm/s

Dose = 7.5 to 30 mg

CYP 3A4 K_m = 4 µM
V_{max} = 850 pmol/min/mg prot.
V_{maxPed} = 561 pmol/min/mg

Paine MF, et al., JPET, 283(3):1552 (1997)

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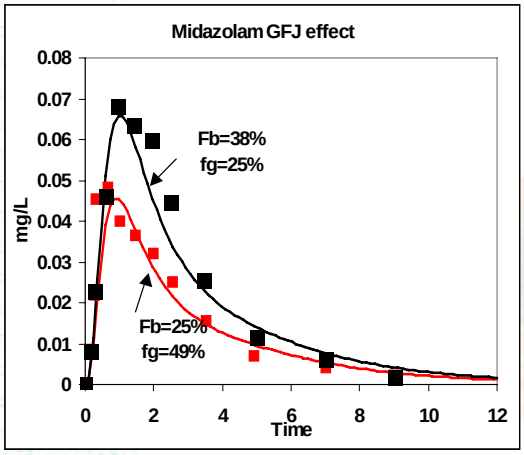
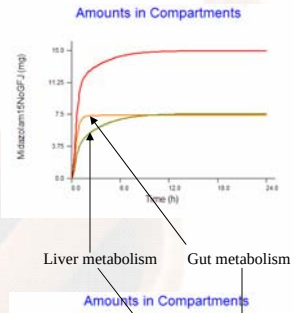
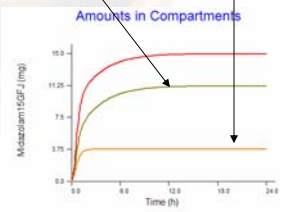
Midazolam



No grapefruit juice





Kupferschmidt HH, Clin. Pharmacol. Therap. 58(1):20 (1995)
Agoram & Bolger, Adv. Drug Deliv. Rev. 50(1):S41 (2001)

After grapefruit juice (gut Vmax reduced by 62%)

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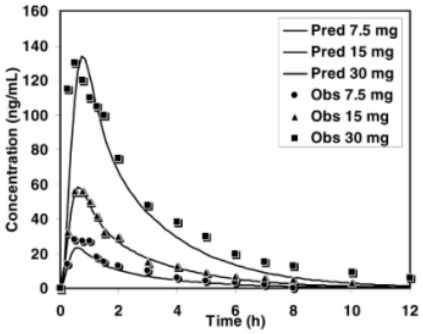
Non-linear Dose Dependence of Midazolam Metabolism in Gut and Liver

Experimental			GastroPlus Compartmental Simulated				
Dose	Cmax	AUC	Cmax	AUC	Fa%	FDP%	Fb%
7.5	0.028	69	0.021	65	99	45	24
15	0.056	154	0.052	158	99	55	29
30	0.13	453	0.120	369	99	64	34

GastroPlus simulations of non-linear dose dependence for Midazolam. (Agoram & Bolger, 2001)



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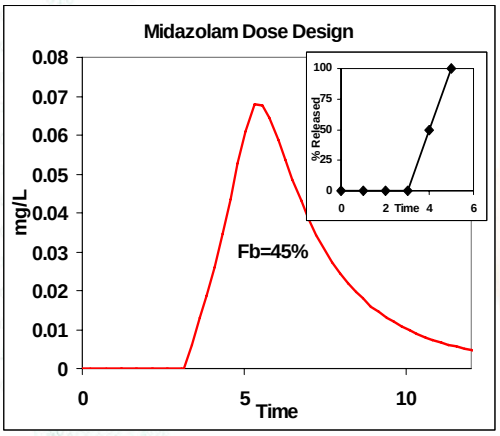
Midazolam (New dose design)



- Aim: To design a new formulation of MDZ to minimize first pass
- Method: Avoid gut metabolism by releasing drug in colon
- 0% released at 3h; 100% released at 5h
- F_b increases from 25% to 45%
- E_g reduces from 49% to 6%

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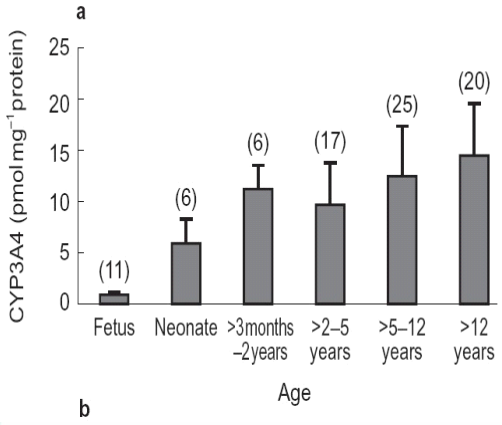
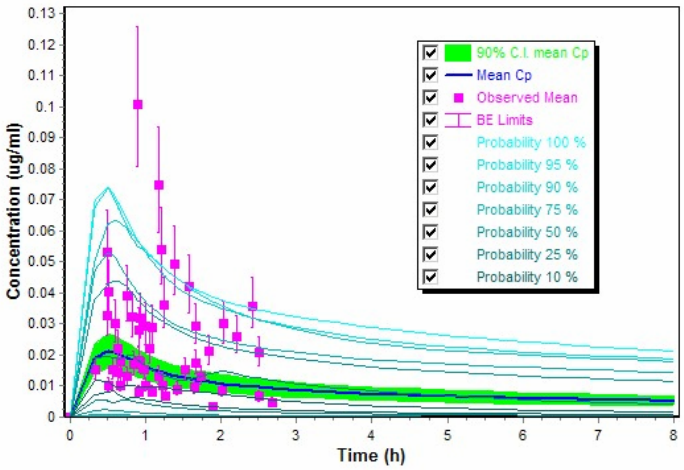
Changes in CYP 3A4 Expression in Duodenum of Pediatric Subjects (1 – 12 yo)



Johnson, T.N., Br. J. Clin. Pharm. 51(5):451 (2001)

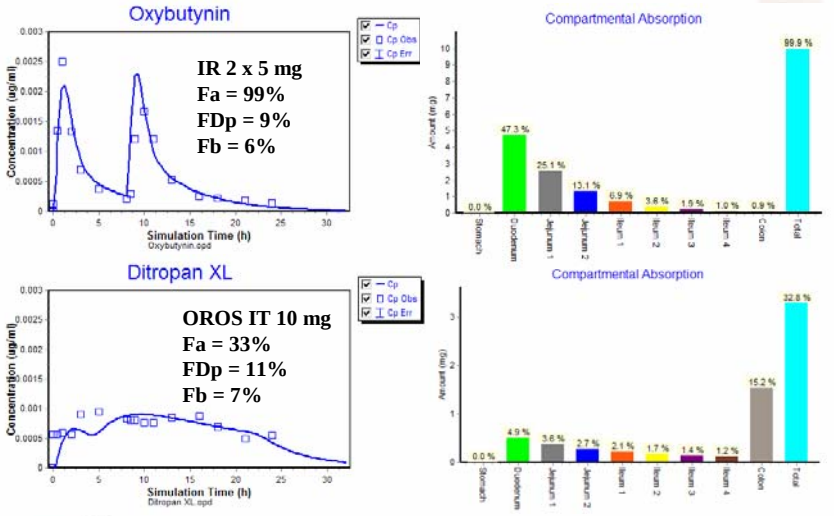
GastroPlus with PBPK module: Pediatric (5 yo) Stochastic population virtual trial:

$V_{max(gut)}$ = 100%, K_m = 50%, SITT = 20%, ColonTT = 20%
Peff = 40%, Other Phys. Params = 10%



Johnson, T.N., Br. J. Anesth. 89(3):428 (2002)

Oxybutynin IR vs CR Integral Tablet



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Sathyan G, Br. J. Clin. Pharm. 52:409 (2001)

Simulations of Non-Linear Influx Transport

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Herrera-Ruiz AAPS Pharmsci 2001; 3 (1) article 9
(http://www.aapspharmaceutica.org)

Human

Peptide Transporter-1

Peptide Transporter-3

Peptide Histidine Transporter 1

Human Peptide Tranporter – 1

β-actin

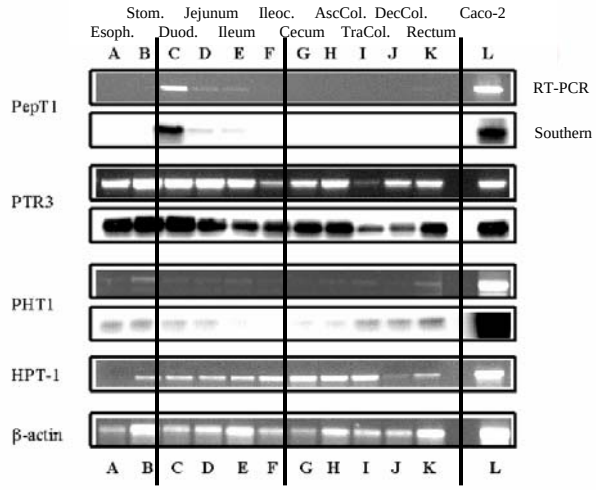

Figure 1. RT-PCR analysis of human PepT1, PTR3, PHT1, and HPT-1 mRNAs in the human esophagus (A), stomach (B), duodenum (C), jejunum (D), ileum (E), ileocecum (F), cecum (G), ascending colon (H), transverse colon (I), descending colon (J), rectum (K), and in Caco-2 cells (L). RT-PCR was performed with specific primers for each mRNA and amplified products of PepT1, PTR3, PHT1, and HPT-1 were 588, 470, 443, and 1004 bp, respectively. Reaction products were electrophoretically separated in 1.4% agarose gels, stained with ethidium bromide (top panels), and identity confirmed by Southern Blot analysis (lower panels). Commercially available human β-actin primers were used to generate a mRNA expression positive control, amplifying a product of 303 bp.

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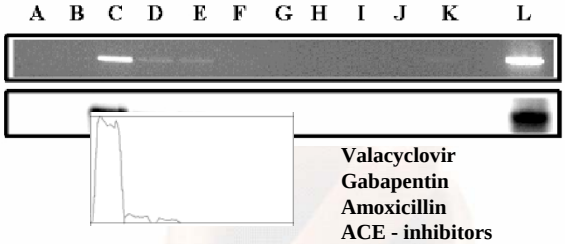
Image J Analysis of Expression

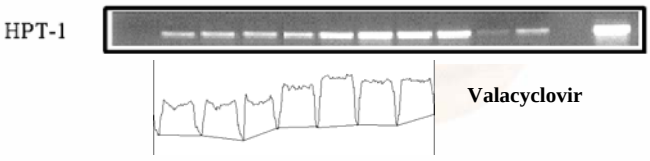
- PepT1

Similar to I



Valacyclovir
Gabapentin
Amoxicillin
ACE - inhibitors

- HPT1



Valacyclovir

Herrera-Ruiz, AAPS Pharmsci 2001; 3 (1) article 9
(http://www.aapspharmaceutica.com)

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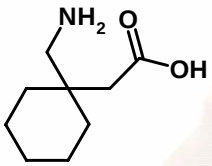
Gabapentin



- Substrate for L-type amino acid transporter (LAT1)
  - Similar distribution to PepT1 (high in duodenum)
- Log P = -1.36 (QP) log $D_{7.0}$ = -2.95 (Exp.)
- Acid pKa = 4.19 and Basic pKa = 10.14 (QP)
- LAT1 IC_{50} = 340 μM (58.2 μg/mL)
- $Solubility_{\sim 7.0}$ = 11.9 mg/mL (QP) 30 mg/mL (Exp.)
- $Peff_{QMPR}$ = 0.8 x 10^{-4} cm/s (Passive Transcellular)
- Renal Clearance

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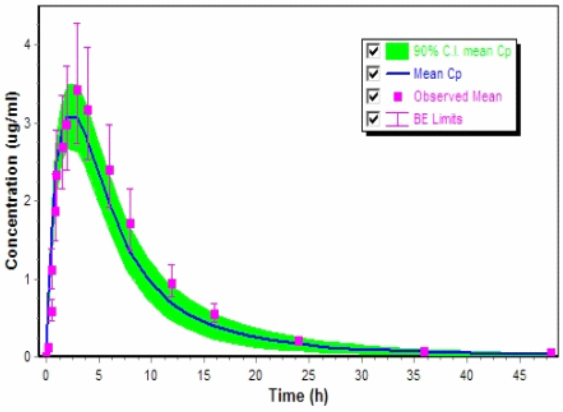
400 mg Solution Dose used to Optimize Compartmental PK

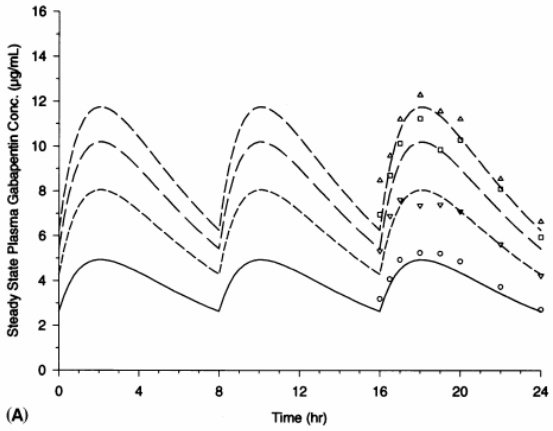


400 mg Solution – 41 yo Female

GPEN, Kansas, 2006

B.E. Gidal et al. / Epilepsy Research 31 (1998) 91–99

Predicted and Observed Steady-state Plasma Gabapentin Concentrations



Predicted Steady-state Plasma Gabapentin Concentrations Following q6h Administration

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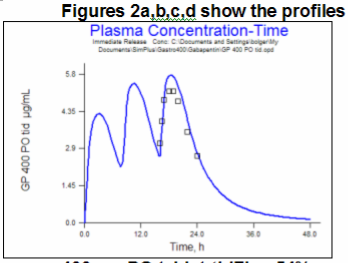
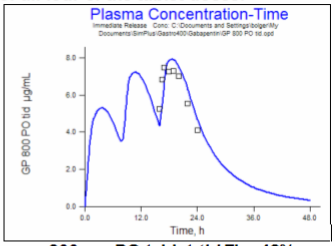
Simulated Non-linear Dose Dependence

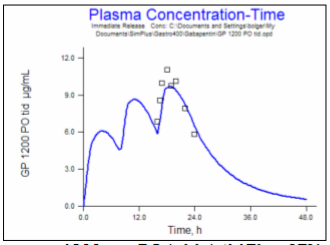
Figures 2a,b,c,d show the profiles for all four of the tablet doses.



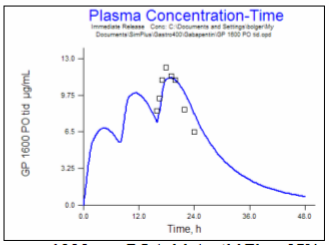
400 mg PO tablet *tid* Fb = 54%



800 mg PO tablet *tid* Fb= 42%



1200 mg PO tablet *tid* Fb = 37%



1600 mg PO tablets *tid* Fb = 35%

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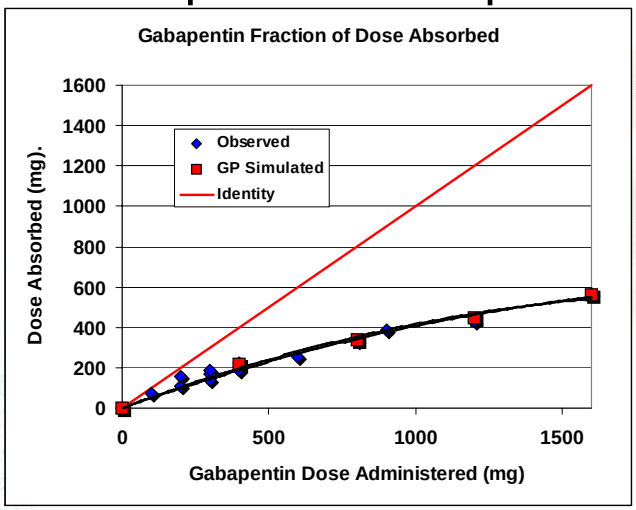
Non-linear Dose Dependence for Gabapentin Absorption



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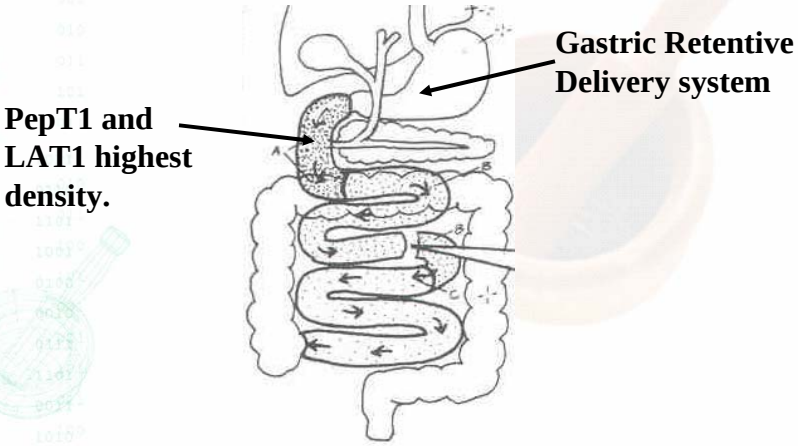
What if we could slowly release Gabapentin with a Gastric Retentive Delivery System once per day?



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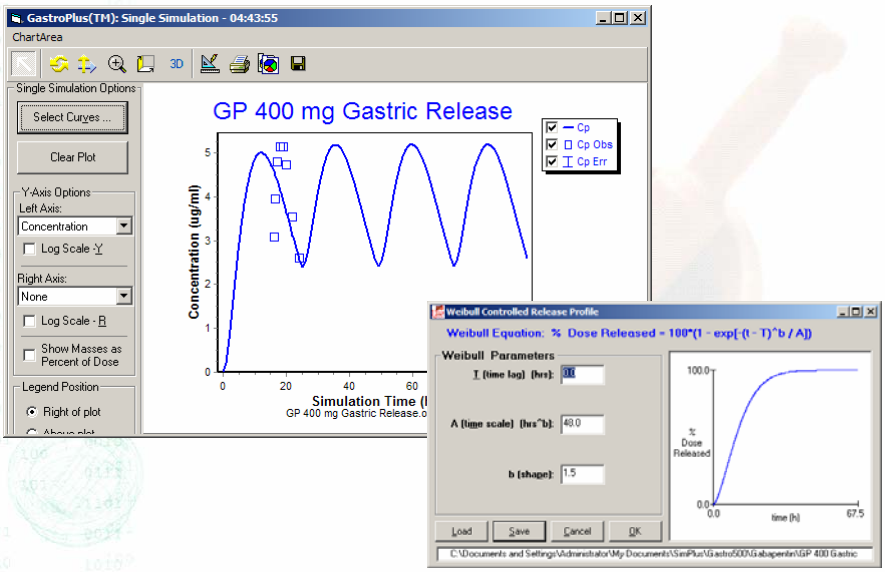
Simulation of Slow Gastric Release



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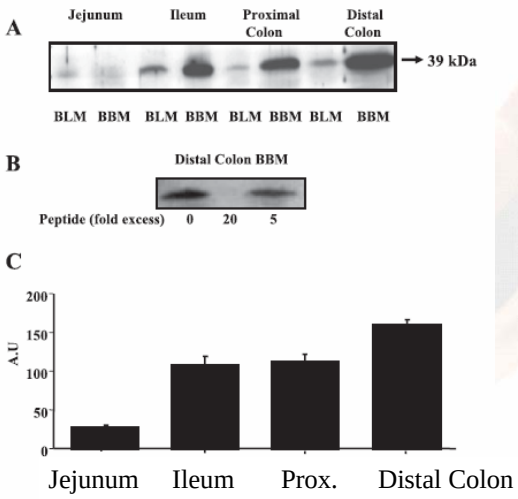
Monocarboxylate Transporter (MCT-1) Expression in Gut



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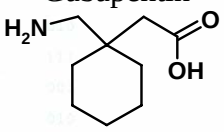
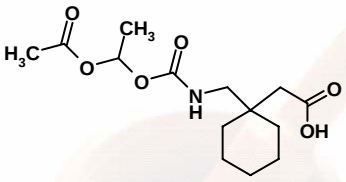
Gill RK, Am. J. Physiol. :Cell Physiol. 289(4):846 (2005)

Gabapentin Prodrug Targeted to MCT1

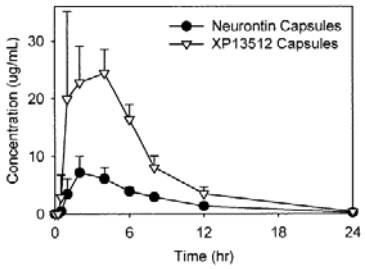
Gabapentin



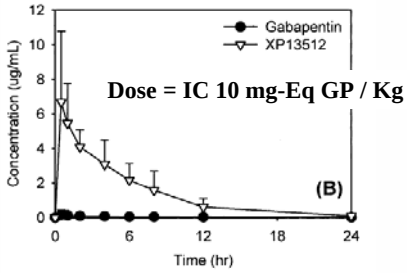
XP13512 (Xenoport)



Dose = IR 36 mg-Eq GP / Kg



Monkey Oral



Monkey Intracolonic

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Cundy KC, JPET 311 (1):324 (2004)

Conclusions

- *In silico* estimates of biopharmaceutical properties are useful in early discovery.
- The combination of *in silico*, *in vitro,* and PBPK provide useful simulations prior to *in vivo* testing.
- Significance / Relevance of transporters can be studied with simulation.
- Data Integration is essential.

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The End

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