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

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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
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:

![Diagram of tissue models with blood and cellular compartments]

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

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

![Diagram of blood compartments with equations]

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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)

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

Kp Calculation Options
**In Silico Tissue Distribution**

- Predicting Tissue/Plasma Partition (Kp):
  - Poulin & Thiel
  
  \[
  K_p = \frac{\left[ K \left( V_{\text{nil}} + 0.3 V_{\text{pht}} \right) + 1 \left( V_{\text{nil}} + 0.7 V_{\text{pht}} \right) \right]}{\left[ K \left( V_{\text{nil}} + 0.3 V_{\text{pht}} \right) + 1 \left( V_{\text{nil}} + 0.7 V_{\text{pht}} \right) \right]} \cdot \frac{\text{fut}_p}{\text{fut}_t}
  \]

  adipose: \( K = D^*_w \)
  other: \( K = P_{ow} \)

  \[
  \log P_{ow} = 1.115 \log P_{ow} - 1.35 \quad \text{Leo, Hansch}
  \]

  \[
  \text{fut}_t = \left[ \frac{1 + (1 - \text{fut}_p)}{\text{fut}_p} \right] \cdot RA_{tp}
  \]

  \( V_{\text{nil}}, V_{\text{pht}}, V_{wt} \): 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:

\[
K_p = Kpu \cdot fup
\]

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 ≥ 7** – takes into consideration the unique interaction of bases with acidic phospholipids (details)

   \[
   Kpu = V_{\text{nil}} + \left( \frac{(1/X_{\text{iw},\text{pht}})}{(1/X_{\text{iw},\text{pht}})} \right) \cdot \left( \frac{K\cdot[AP]_T}{(1/X_{\text{iw},\text{pht}})} \right) \cdot \left( \frac{\text{fup} \cdot (0.3K + 0.7)V_{\text{pht}}}{(1/X_{\text{iw},\text{pht}})} \right)
   \]

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

   \[
   Kpu = \left( \frac{(1/X_{\text{iw},\text{pht}})}{(1/X_{\text{iw},\text{pht}})} \right) V_{\text{nil}} + \left( \frac{K\cdot[V_{\text{pht}} + (0.3K + 0.7)V_{\text{pht}}]}{\left(1/X_{\text{iw},\text{pht}}\right)} \right) \cdot \left[ \frac{1}{\text{fup} \cdot (1 - K\cdot[V_{\text{pht}} + (0.3K + 0.7)V_{\text{pht}}]} \right] \cdot RA_t
   \]

\( X_{\text{iw}} \) – fraction of neutral drug species in intracellular water (IW, pH=7) and plasma (P, pH=7.4)

\( K \) – vegetable oil/water partition coefficient for adipose tissue and 1-octanol/water partition coefficient for remaining tissues

\( fup \) – fraction unbound of drug in plasma, \( Kpu \) – association constant of base with acidic phospholipids, \( [AP]_T \) – 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.

Enterocyte Model for Each Compartment

Hepatic Artery 375 mL/min

Portal Vein 1125 mL/min

Circulation

Renal Excretion

Gastrointestinal Cell (Enterocyte)

Efflux

Plasma Protein Binding

Liver Enzymes

Metabolism

Drug

Gastrointestinal Cell (Enterocyte)

Peff

Gl

Gastrointestinal Lumen

Feces Excretion

Gastrointestinal Cell (Enterocyte)


taken from

GPEN, Kansas, 2006
**Carrier-mediated Transport**

\[
dM_{\text{ent}(i)}/dt =
\]
\[
\text{Apical Diffusion Rate} + \text{Apical Carrier-mediated Transport Rate} - \text{Basolateral Transfer Rate} - \text{Gut Metabolism Rate}
\]

Apical Carrier-mediated Transport rate =
\[
DF_{\text{influx}(i)} V_{\text{max},\text{influx}} C_{(i)} / (K_{m,\text{influx}} + C_{(i)})
\]
\[
- DF_{\text{efflux}(i)} V_{\text{max},\text{efflux}} C_{\text{u,ent}(i)} / (K_{m,\text{efflux}} + C_{\text{u,ent}(i)})
\]

DF = distribution factor for transporter amounts relative to \(V_{\text{max}}\) measurement environment (when \(V_{\text{max}}\) in a compartment is the same as \(V_{\text{max}}\) 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**

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

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

3. Based on a hepatic blood flow model (e.g. Venous equilibrium model), determine *in vivo* hepatic clearance. Rate of drug elimination = $\text{CL}_h \times \text{Concentration}$.
Significance of Gut Metabolism and Controlled Release

Gut Metabolism Scale Factors

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

3A4 (nmol) = 9.7 38.4 22.4
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

GPEN, Kansas, 2006

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

Midazolam
$V_{max} = 850$ pmol/min/mg
$K_m = 4 \mu M$
$V_{max}/K_m = 212$
IR Intest. Extract. = 43%

Alprazolam
$V_{max} = 2680$ pmol/min/mg
$K_m = 660 \mu M$
$V_{max}/K_m = 4.1$
IR Intest. Extract. ~ 1%

Saquinavir
$V_{max} = 3960$ pmol/min/mg
$K_m = 0.4 \mu M$
$V_{max}/K_m = 9900$
IR Intest. Extract. = 64%

Fitzsimmons-DrugMetabDisp-25-2-255-1997-SaquinavirMetabolismIntestine.pdf
GPEN, Kansas, 2006
**Intestinal and Hepatic CYP 3A4 Metabolism**

**Midazolam**

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 Km = 4 μM
Vmax = 850 pmol/min/mg prot.
VmaxPed = 561 pmol/min/mg

Midazolam GFJ effect


After grapefruit juice (gut Vmax reduced by 62%)

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

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

<table>
<thead>
<tr>
<th>Experimental</th>
<th>GastroPlus Compartimental Simulated</th>
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<tbody>
<tr>
<td>Dose</td>
<td>Cmax</td>
</tr>
<tr>
<td>7.5</td>
<td>0.028</td>
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<tr>
<td>15</td>
<td>0.056</td>
</tr>
<tr>
<td>30</td>
<td>0.13</td>
</tr>
</tbody>
</table>

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%
GPEN, Kansas, 2006

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


GastroPlus with PBPK module:
Pediatric (5 yo) Stochastic population virtual trial:
\[ V_{\text{max(gut)}} = 100\%, K_m = 50\%, SITT = 20\%, \text{ColonTT} = 20\% \]
\[ Peff = 40\%, \text{Other Phys. Params} = 10\% \]

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**Oxybutynin IR vs CR Integral Tablet**

**Oxybutynin**

IR 2 x 5 mg
Fa = 99%
FDp = 9%
Fb = 6%

**Ditropan XL**

OROS IT 10 mg
Fa = 33%
FDp = 11%
Fb = 7%


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**Simulations of Non-Linear Influx Transport**

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**Peptide Transporter-1**

**Peptide Transporter-3**

**Peptide Histidine Transporter 1**

**Human Peptide Transporter – 1**

**β-actin**


**β-actin**

Herrera-Ruiz AAPS PharmSci 2001; 3 (1) article 9

**Image J Analysis of Expression**

- **PepT1** Similar to LAT1

- **HPT1**

Valacyclovir Gabapentin Amoxicillin ACE - inhibitors

Herrera-Ruiz, AAPS PharmSci 2001; 3 (1) article 9
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 \( \mu \)M (58.2 \( \mu \)g/mL)
- Solubility\(_{7.0}\) = 11.9 mg/mL (QP)  30 mg/mL (Exp.)
- \( Peff_{\text{QMPPR}} = 0.8 \times 10^{-4} \) cm/s (Passive Transcellular)
- Renal Clearance

400 mg Solution Dose used to Optimize Compartmental PK

400 mg Solution – 41 yo Female
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

![Graph showing Gabapentin Fraction of Dose Absorbed vs Gabapentin Dose Administered (mg)]

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

Gastric Retentive Delivery system

PepT1 and LAT1 highest density.

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Simulation of Slow Gastric Release

Monocarboxylate Transporter (MCT-1) Expression in Gut

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