Biochemical and Molecular Toxicology
ENVR 442/TOXC 442/BIOC 442

Principles of
Toxicokinetics/Toxicodynamics

Kim L.R. Brouwer, PharmD, PhD
kbrouwer@unc.edu; 919-962-7030
Pharmacokinetics/
Toxicokinetics:

the study of the time course of xenobiotic absorption, distribution, metabolism and excretion
ADME

- **Absorption**
  - how the xenobiotic enters the body

- **Distribution**
  - where the xenobiotic goes in the body

- **Metabolism**
  - what the body does to the xenobiotic

- **Elimination**
  - how the xenobiotic is removed from the body
Pharmacodynamics/Toxicodynamics:

the relationship between xenobiotic concentration at the site of action and the resulting effect, including the time course and intensity of therapeutic and adverse effects
Pharmacokinetics/Toxicokinetics

Dosage Regimen/Exposure → Plasma Concentration

Effects: Pharmacologic Toxic

Pharmacodynamics/Toxicodynamics

Site of Action
Toxicokinetic/ADME Studies

- Pharmacokinetics / Bioavailability
- Mass Balance
- Tissue Distribution
- Metabolite Profile
- Plasma Protein Binding
- Inhibition / Induction
Terms used in Pharmacokinetics/Toxicokinetics:

- $C_{\text{max}}$
- $T_{\text{max}}$
- $\lambda$
- $t_{1/2}$
- AUC
- AUMC
- $V_{D_{ss}}$
- $f_u$
- $\text{Cl}$
- MRT
- MAT
- $F$
- $C_{\text{max}}$
  maximum concentration of compound observed in the matrix of interest
- $T_{\text{max}}$
  time of maximum concentration
$C_{\text{max}}$ - time of maximum concentration

$T_{\text{max}}$ - time of maximum concentration
- **lambda** ($\lambda$)
  terminal elimination rate constant
  (slope from a semi-log concentration vs time plot)

- **$t_{1/2}$**
  half-life: the time it takes for the concentration of the compound to decrease by 50%

\[ t_{\frac{1}{2}} = \frac{\ln 2}{\lambda} \]

- slope = \( \lambda \)
The Half-life is Compound- and Subject-Dependent

<table>
<thead>
<tr>
<th>Compound</th>
<th>Name</th>
<th>$t_{1/2}$ (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rosiglitazone</td>
<td>Avandia</td>
<td>3-4</td>
</tr>
<tr>
<td>Lamotrigine</td>
<td>Lamictal</td>
<td>25-33</td>
</tr>
<tr>
<td>Buproprion</td>
<td>Wellbutrin</td>
<td>21</td>
</tr>
<tr>
<td>Fluticasone</td>
<td>Flovent</td>
<td>n/a</td>
</tr>
<tr>
<td>Paroxetine</td>
<td>Paxil</td>
<td>21</td>
</tr>
</tbody>
</table>

Data from LexiComp
Half-life

- Half-life is used to:
  - Determine the time to reach steady-state \((5 \times t_{1/2})\)
  - Determine the time to eliminate all the compound from the body \((5 \times t_{1/2})\)
  - Calculate dosing intervals
  - Determine how much compound accumulates in the body given a fixed dosing interval or exposure

- Half-life is a secondary pharmacokinetic parameter that is determined by the volume of distribution \((V)\) and clearance \((Cl)\) of the compound

\[
t_{1/2} = \frac{0.693 \times V}{Cl}
\]
AUC

area under the concentration vs time curve
\[ \text{AUC}_{0\to\infty} = \text{AUC}_{\tau} \]

- Single Dose
- Multiple Dose to Steady-State
Steady-state

- At Steady-State:
  
  Rate of input = Rate of elimination
- **AUMC**

  area under the first moment curve
Mean Residence Time (MRT)

- The average time one molecule resides in the body
- MRT = AUMC/AUC
- Used to express overall persistence of compound in the body
- Clearance (Cl)
  - volume of fluid (usually blood) from which compound is removed completely per unit time

Mathematically:

\[ Cl = \frac{Dose}{AUC_\infty} \]

- Organs that may be involved in clearance:
  - GI tract
  - Liver
  - Kidney
  - Lungs
  - Other sites (e.g., blood, skin)
Clearances (Cl) are Additive
Pharmacokinetic Parameters of GI158104X in Dog

<table>
<thead>
<tr>
<th>Dose mg/kg/day</th>
<th>$\text{Cl}_{\text{IV}}$ (mL/min/kg)</th>
<th>$\text{Cl}_r$ (mL/min/kg)</th>
<th>$\text{Cl}_{\text{other}}$ (mL/min/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>9.5</td>
<td>10</td>
<td>0.7</td>
<td>9.3</td>
</tr>
</tbody>
</table>
Linear Pharmacokinetics

- Linear Kinetics = Dose-Proportional kinetics
- AUC (or concentration) changes proportionally with dose
Pharmacokinetics and Drug Metabolism: Maximum plasma concentration ($C_{\text{max}}$) and the area under the curve (AUC) of rosiglitazone increase in a dose-proportional manner over the therapeutic dose range (see Table 1). The elimination half-life is 3 to 4 hours and is independent of dose.

Table 1. Mean (SD) Pharmacokinetic Parameters for Rosiglitazone Following Single Oral Doses ($N = 32$)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>1 mg Fasting</th>
<th>2 mg Fasting</th>
<th>8 mg Fasting</th>
<th>8 mg Fed</th>
</tr>
</thead>
<tbody>
<tr>
<td>$AUC_{0-\text{inf}}$ [ng•hr/mL]</td>
<td>358 (112)</td>
<td>733 (184)</td>
<td>2,971 (730)</td>
<td>2,890 (795)</td>
</tr>
<tr>
<td>$C_{\text{max}}$ [ng/mL]</td>
<td>76 (13)</td>
<td>156 (42)</td>
<td>598 (117)</td>
<td>432 (92)</td>
</tr>
</tbody>
</table>
Stationary Pharmacokinetics

- Pharmacokinetic parameters are independent of time

Package insert for Tegretol (carbamazepine)

Because Tegretol induces its own metabolism, the half-life is also variable. Autoinduction is completed after 3-5 weeks of a fixed dosing regimen. Initial half-life values range from 25-65 hrs, decreasing to 12-17 hrs on repeated doses. Tegretol is metabolized in the liver.
Volume of Distribution at Steady-State ($V_{d_{ss}}$)

a parameter that relates plasma concentration to total mass of compound in the body

Mathematically:

$$Vd_{ss} = \frac{Dose \times AUMC}{AUC^2}$$
The Volume of Distribution is Compound- and Subject-Dependent

<table>
<thead>
<tr>
<th>Compound</th>
<th>Name</th>
<th>V (L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rosiglitazone</td>
<td>Avandia</td>
<td>18</td>
</tr>
<tr>
<td>Lamotrigine</td>
<td>Lamictal</td>
<td>70</td>
</tr>
<tr>
<td>Bupropion</td>
<td>Wellbutrin</td>
<td>140</td>
</tr>
<tr>
<td>Fluticasone</td>
<td>Flovent</td>
<td>280</td>
</tr>
<tr>
<td>Paroxetine</td>
<td>Paxil</td>
<td>560</td>
</tr>
</tbody>
</table>

Data from LexiComp
- Fraction Unbound ($f_u$)
  - fraction of drug that is not bound to plasma proteins
  - the unbound concentration is in equilibrium between the tissues and blood
Protein-Binding Changes May be Clinically Relevant for the Following Types of Compounds and Routes of Administration

<table>
<thead>
<tr>
<th></th>
<th>High Extraction Ratio</th>
<th>Low Extraction Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>IV Administration</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hepatic Clearance</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Nonhepatic Clearance</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Oral Administration</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hepatic Clearance</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Nonhepatic Clearance</td>
<td>Yes†</td>
<td>No</td>
</tr>
</tbody>
</table>

† No drugs from a list of 456 met these criteria

*Benet and Hoener, Clin Pharmacol Ther 71:115, 2002*
Organ Extraction (E)

\[ E = \frac{C_{in} - C_{out}}{C_{in}} \]
When Is Protein Binding Important?

- *In vitro* ADME/preclinical toxicology studies
- Scaling pharmacokinetic/pharmacodynamic parameters from animal models to humans
- Calculating human doses from *in vitro* measures of target concentrations
- Therapeutic drug monitoring of blood/plasma concentrations for drugs with a narrow therapeutic index
• **Bioavailability (F)**

  fraction of the administered dose that reaches the systemic circulation intact

  \[ 0 \leq F \leq 1 \]

  • **Mathematically:**

  \[
  F = \frac{AUC_{PO}}{AUC_{IV}} \ast \frac{Dose_{IV}}{Dose_{PO}}
  \]
Relative Bioavailability

fraction of the dose of a test product that reaches the systemic circulation relative to a reference product

Mathematically:

$$F = \frac{AUC_{test}}{AUC_{ref}} \times \frac{Dose_{ref}}{Dose_{test}}$$
Bioavailability is an important determinant of pharmacologic response and therapeutic effectiveness.
Estimation of Oral Bioavailability (F) After an IV and Oral Dose of Compound X

<table>
<thead>
<tr>
<th>Dose mg/kg/day</th>
<th>( \text{AUC}_{\text{IV}} ) (^1) (( \mu \text{g} \cdot \text{hr}/\text{mL} ))</th>
<th>( \text{AUC}_{\text{PO}} ) (^1) (( \mu \text{g} \cdot \text{hr}/\text{mL} ))</th>
<th>( F )</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.0</td>
<td>6.25</td>
<td>5.94</td>
<td>0.95</td>
</tr>
<tr>
<td>25.0</td>
<td>32.4</td>
<td>8.15</td>
<td>0.25</td>
</tr>
</tbody>
</table>

\(^1\) mean, n=4
Factors Responsible for Poor or Variable Oral Bioavailability

- Physicochemical Factors
- Physiologic Factors
Influence of Formulation Factors on the Concentration-Time Profile

- Formulations with different release rates (absorption kinetics)
Influence of Formulation on the Bioavailability of a Compound

- Bioequivalence studies are performed to select the formulation which gives the greatest oral exposure to the drug.
Special Studies: Effects of Food on Exposure

- Often the fed/fasted state of the animal can influence the absorption of drugs
- Food can enhance or inhibit absorption
- Can impact on clinical dosing
Physiologic Factors Responsible for Poor or Variable Oral Bioavailability

- Cell Membranes
- pH, Volume and Content of GI Fluid
- GI Motility
- Vascularity and Blood Flow
- Enzymatic Degradation / Metabolism
- Disease States
The Gastrointestinal Tract
Presystemic Elimination: (First-Pass Effect)
Loss or metabolism of drug between the administration site and the sampling site
Factors Affecting Systemic Availability

- Gut Lumen
- Gut Wall
- Portal Vein
  - Protein Binding
  - RBC Partitioning
  - Blood/Plasma Stability
- Systemic Circulation
- Liver
- Metabolism
- Biliary Excretion

Factors:
- Solubility
- Chemical Stability
- Permeability
- Mucosal Metabolism
- Transport/Efflux
- Metabolism
- Feces
- Protein Binding
- RBC Partitioning
- Blood/Plasma Stability
- Systemic Circulation
First-pass Intestinal and Hepatic Metabolism of Cyclosporin

Benet et al., J Controlled Release 39:139, 1996
In Vivo Pharmacokinetic and Bioavailability Studies

- Resulting Information:
  - Pharmacokinetic parameters ($t_{1/2}$, AUC, $V_{dss}$, Cl, F) in the species used for the multiple dose toxicology studies
  - Rate and extent of drug absorption
  - Primary routes of elimination

- Utility:
  - Identifies bioavailability problems
  - Parameters are used in scaling to humans to predict the initial human dose
Additional Studies for Compounds That Exhibit Poor Bioavailability

- *In Vivo* Studies to Assess Sites of Presystemic Elimination:

\[
F_{\text{Organ(s)}} = \frac{AUC_{\text{Pre–Organ(s)}}}{AUC_{\text{IV}}} \times \frac{Dose_{\text{IV}}}{Dose_{\text{Pre–Organ(s)}}}
\]
Assessing Sites of Presystemic Elimination

- Dose to Stomach
- Dose to Duodenum
- Dose to Jejunum & Ileum
- Portal Vein
- Liver
- Systemic Circulation
Additional Studies for Compounds That Exhibit Poor Bioavailability

• In Vivo Portal Vein Administration Studies:

\[
F_{PV} = \frac{AUC_{PV}}{AUC_{IV}} \times \frac{Dose_{IV}}{Dose_{PV}}
\]

– If \( F_{PV} \) approximates 100%, presystemic elimination occurs prior to the portal vein (gut lumen or gut wall)

– If \( F_{PV} \) approximates \( F_{PO} \), presystemic elimination occurs primarily in the liver
Assessing Sites of Presystemic Elimination

- Stomach
- Duodenum
- Jejunum & Ileum
- Portal Vein
- Liver
- Systemic Circulation
## Case Study: Assessing Sites of Presystemic Elimination

<table>
<thead>
<tr>
<th>Route of Administration</th>
<th>AUC (µg*hr/ml)</th>
<th>Bioavailability (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oral</td>
<td>972</td>
<td>21</td>
</tr>
<tr>
<td>Intraduodenal</td>
<td>1378</td>
<td>30</td>
</tr>
<tr>
<td>Portal Vein</td>
<td>4386</td>
<td>94</td>
</tr>
<tr>
<td>Intravenous</td>
<td>4653</td>
<td>100</td>
</tr>
</tbody>
</table>
In Situ and In Vitro Models to Assess Sites of Presystemic Elimination

- Models to Assess Presystemic Elimination Prior to the Portal Vein
  - Isolated Perfused Intestinal Segments
  - Everted Gut Sacs
  - Intestinal Rings
  - Cell Monolayers

- Models to Assess Hepatic Presystemic Elimination
  - Isolated Perfused Liver
  - Liver Slices
  - Hepatocytes (freshly isolated/cultured)
Toxicokinetics

- Dose Selection
- Dose Proportionality
- Multiple Dose Pharmacokinetics
- Induction/Inhibition
- Exposure Verification
Toxicokinetics: Dose Selection

- Determination of the optimum dose
  - primarily focused on the relationship between dose and exposure
    » linearity
    » saturation
**$C_{\text{max}}$ and AUC Following Escalating Doses of Compound A**

<table>
<thead>
<tr>
<th>Dose mg/kg/day</th>
<th>$C_{\text{max}}$ $^1$ (ug/mL)</th>
<th>AUC $^1$ (ug*hr/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.0</td>
<td>1.40</td>
<td>6.25</td>
</tr>
<tr>
<td>25.0</td>
<td>9.47</td>
<td>32.4</td>
</tr>
<tr>
<td>100.0</td>
<td>10.56</td>
<td>47.2</td>
</tr>
</tbody>
</table>

$^1$ mean, n=4
Dose Selection

![Graph showing AUC vs. Dose for Rat and Dog](image-url)
Saturation of Absorption

![Graph showing bioavailability vs dose for mouse and dog.]

- **Bioavailability (%)**
- **Dose (mg/kg)**

Mouse:
- Dose 25, Bioavailability 35%
- Dose 500, Bioavailability 5%

Dog:
- Dose 10, Bioavailability 40%
- Dose 500, Bioavailability 10%
Toxicokinetics: Dose Proportionality

A = AUC increases proportional to dose.

B = Less than proportional increase in exposure: Absorption-limited exposure.

C = Greater than proportional increase in exposure: Capacity-limited elimination

Interpretation of results demonstrating lower toxicity at higher doses depends on whether compound exhibits dose-proportional kinetics.
Toxicokinetics: Multiple Dose Pharmacokinetics

- Determine how drug distribution is altered after multiple dosing
  - Examine changes in clearance, half-life, accumulation, linearity
  - Assure dose related continuous exposure of the animals to the test compound

- Evaluate:
  - Toxicokinetic changes with dose and time
  - Effect of advancing age of the animals
  - Decreases in lean body mass, total body water, cardiac output, renal, hepatic and GI tract blood flow
Terminal $t_{1/2}$ (mean and SD) on Days 1 and 30 During a Rat 1-Month Oral Multiple Dosing Study

<table>
<thead>
<tr>
<th>Dose (mg/kg/day)</th>
<th>Day 1</th>
<th>Day 30</th>
<th>Dose (mg/kg/day)</th>
<th>Day 1</th>
<th>Day 30</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>2.94</td>
<td>2.31</td>
<td>5</td>
<td>2.15</td>
<td>1.72</td>
</tr>
<tr>
<td></td>
<td>0.86</td>
<td>0.29</td>
<td></td>
<td>0.10</td>
<td>0.09</td>
</tr>
<tr>
<td>25</td>
<td>2.18</td>
<td>2.06</td>
<td>25</td>
<td>2.45</td>
<td>1.99</td>
</tr>
<tr>
<td></td>
<td>0.30</td>
<td>0.25</td>
<td></td>
<td>1.07</td>
<td>0.39</td>
</tr>
<tr>
<td>150</td>
<td>15.33</td>
<td>2.14</td>
<td>150</td>
<td>9.76</td>
<td>2.72</td>
</tr>
<tr>
<td></td>
<td>3.32</td>
<td>0.14</td>
<td></td>
<td>4.54</td>
<td>0.71</td>
</tr>
</tbody>
</table>
Effect of Age on the Toxicokinetics of N-Acetylprocainamide HCl After IV Administration of 100 mg/kg in Rats

<table>
<thead>
<tr>
<th>Parameters</th>
<th>13 Weeks Mean</th>
<th>(n=7) CV%</th>
<th>26 Weeks Mean</th>
<th>(n=11) CV%</th>
<th>52 Weeks Mean</th>
<th>(n=7) CV%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Half-life (hr)</td>
<td>1.66</td>
<td>12.1</td>
<td>1.82</td>
<td>14.2</td>
<td>2.29</td>
<td>42.3</td>
</tr>
<tr>
<td>(C_0) (ug/mL)</td>
<td>19.0</td>
<td>12.1</td>
<td>27.7</td>
<td>21.6</td>
<td>46.9</td>
<td>21.8</td>
</tr>
<tr>
<td>AUC (ug*hr/mL)</td>
<td>45.3</td>
<td>18.2</td>
<td>72.6</td>
<td>27.0</td>
<td>156.0</td>
<td>51.4</td>
</tr>
<tr>
<td>Cl (L/hr/kg)</td>
<td>2.01</td>
<td>14.8</td>
<td>1.29</td>
<td>23.5</td>
<td>0.664</td>
<td>71.4</td>
</tr>
<tr>
<td>Vd (L/kg)</td>
<td>4.75</td>
<td>11.2</td>
<td>3.35</td>
<td>22.0</td>
<td>1.98</td>
<td>24.9</td>
</tr>
</tbody>
</table>

From: S. Garattini, Drug Metabolism Reviews 13, (3), 1982
Time (Exposure) Dependence

- Induction and Inhibition of metabolism as examples of elimination changes
- Induction or Inhibition may occur without affecting the overall disposition of the test compound
Exposure Verification - example

- Continuous IV infusion for 14 days in rats and dogs
- Infusion solution at pH = 5
- Blood samples taken at various times to document exposure
- Pilot (24 hr) study in both species
Special Toxicokinetic Issues

- Toxicity / Potency Evaluations
- Metabolic Sites
- Correlation of Residual Drug with Toxicity
- Species Selection
- Gender Effects
- Placental Transfer
- Milk Transfer
Coverage for Human Metabolites

- **Species Selection**
  - confirmation that the species chosen for oncogenicity studies supports the human metabolism
  - is there coverage in the animal species for all metabolites generated in human systems

Cumulative Percent of Radioactive Dose in Urine and Feces of Male Cynomolgus Monkeys After a Single IV Dose of $[^{14}\text{C}]\text{GW280430}$

<table>
<thead>
<tr>
<th>Collection Interval (Hours)</th>
<th>Animal Number</th>
<th>Urine</th>
<th>Feces</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>I04646</td>
<td>I04647</td>
<td>I04650</td>
</tr>
<tr>
<td>----------------------------</td>
<td>---------------</td>
<td>-------</td>
<td>-------</td>
</tr>
<tr>
<td>0-2</td>
<td>14.5</td>
<td>4.16</td>
<td>25.1</td>
</tr>
<tr>
<td>0-6</td>
<td>42.6</td>
<td>31.2</td>
<td>40.4</td>
</tr>
<tr>
<td>0-12</td>
<td>43.3</td>
<td>35.2</td>
<td>45.0</td>
</tr>
<tr>
<td>0-24</td>
<td>46.2</td>
<td>37.7</td>
<td>49.1</td>
</tr>
<tr>
<td>0-48</td>
<td>48.1</td>
<td>40.0</td>
<td>51.0</td>
</tr>
<tr>
<td>0-72</td>
<td>49.2</td>
<td>41.0</td>
<td>51.8</td>
</tr>
<tr>
<td>0-96</td>
<td>50.0</td>
<td>42.1</td>
<td>52.4</td>
</tr>
<tr>
<td>0-120</td>
<td>50.8</td>
<td>42.9</td>
<td>52.9</td>
</tr>
<tr>
<td>0-144</td>
<td>51.9</td>
<td>43.6</td>
<td>53.1</td>
</tr>
<tr>
<td>0-168</td>
<td>52.3</td>
<td>43.9</td>
<td>53.5</td>
</tr>
<tr>
<td>ND</td>
<td>Not detectable.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SD</td>
<td>Standard deviation.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

ND  Not detectable.
SD  Standard deviation.
Use of WBA to Assess Effect of GF120918 on Antiviral Distribution in Rats

Antiviral Alone

Antiviral + GF120918

Brain/Blood

CSF/Blood

Ratio

0.35
0.86
0.06
0.45
0.00
0.10
0.20
0.30
0.40
0.50
0.60
0.70
0.80
0.90

Ratio

Brain/Blood

CSF/Blood

0.35
0.45
0.06
0.86

Courtesy of Glaxo, Inc.
Toxicokinetic Modeling

- Classic Compartmental Modeling
  - 1-Compartment Model
  - Multi-compartment Model

- Physiologic Compartmental Modeling
  - Perfusion-Limited Compartments
  - Diffusion-Limited Compartments
  - Specialized Compartments
Disposition of Pafuramidine/Furamidine in Rat Isolated Perfused Liver (IPL) and Sandwich-Cultured Hepatocytes (SCH)

**IPL**

- Initial concentration: 10 μM
- Pafuramidine (Perfusate/Medium)
- M3 (Perfusate/Medium)
- Furamidine (Liver/Cells)
- Furamidine (Perfusate/Medium)

**SCH**

- Furamidine (Perfusate/Medium)
- 99%

Perfusate: n = 3-6 rats / timepoint
Liver: n=1 rat / timepoint

Medium & Cells: n = 3 rats / timepoint

Scheme Describing Disposition of Prodrug and Metabolite in Isolated Perfused Liver and Sandwich-Cultured Hepatocytes

Intrahepatic Binding Markedly Influences Disposition of Active Metabolite

- Hepatic accumulation of active metabolite was extensive (>95% of total formed)
- Hepatic unbound fraction ($f_{u,L}$) of furamidine was only 0.3% explaining, at least in part, low perfusate (systemic) exposure of furamidine.

Schematic Structure of Physiologic Toxicokinetic Model for Inhaled Propylene Oxide