Introduction to Pharmacokinetics

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Drug Discovery
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History’s first human drug metabolism experiment

**Early 1800s**

Friedrich Woehler

Chemical transformations in vivo (biotransformation)

Alexander Ure

Gout treatment

W. Keller

Human subject

Benzoic acid + Glycine → Hippuric acid

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In Keller's words: "In the evening, shortly before going to bed, I ingested 2 grams (about 32 grains) of benzoic acid in a sugar syrup. During the night I started sweating, which could be an effect of this acid, since I otherwise very rarely sweat profusely. I experienced no other obvious effect, even over the course of the following days when I took the same dose, and neither did the sweating reoccur. In the urine eliminated the next morning was found to be unusually acidic, even after being allowed to stand 12 hours after evaporation...after the residue was mixed with hydrochloric acid and allowed to stand, a large amount of long prismatic brown crystals was formed, which did look like benzoic acid. Another portion, which had been concentrated to a syrupy thickness, formed a magma of crystalline plates after being mixed with hydrochloric acid...Long, colorless prismatic crystals were then isolated. These crystals consisted of pure hippuric acid. ...As long as I continued to ingest benzoic acid, I could very easily continue to produce quantities of hippuric acid, without any apparent unhealthy effects, so it would be quite easy to produce in such a way great quantities of hippuric acid. One could keep a subject on hand for weeks, in order to continue this method of production."
• **What is pharmacokinetics (PK)?**

• Why do we need PK?

• How do we do PK?

• How is the data generated?

• How do we do PK analysis?
PHARMACOMETRICS

Quantitative description of pharmacology

Science: Pharmacology

Models: Pharmacokinetics, Pharmacodynamics

Experiment: Dose, Concentration, Effect
DEFINITIONS

Pharmacokinetics describes the movement (Greek – kinesis) of a drug (Greek – pharmakon) around the body.

*Pharmacokinetics* is the study of the rates of absorption, distribution, metabolism and excretion of a drug and its metabolite(s). Methods such as Statistical Moments, Sums of exponential modeling and Physiologically based kinetic modeling are used. *Toxicokinetics* is pharmacokinetics studied at high doses (toxic?).

*Clinical Pharmacokinetics* is the application of pharmacokinetic principles to the therapeutic management of patients.

*Population Pharmacokinetics* uses advanced statistical methods and fragmented and sparsely available data to determine PK parameters and their associated variability.
• What is pharmacokinetics (PK)?
• **Why do we need PK?**
• How do we do PK?
• How is the data generated?
• How do we do PK analysis?
Pharmacokinetics tries to answer the questions:

- Why does only a fraction of the total dose reach its target?
- How should we dose (route) and how many times (frequency) to maintain drug at target (efficacy)?
PRE-CLINICAL OUTCOMES FROM DOING PK

• Select compounds that have the maximum potential of reaching the target (PK)
• Select the appropriate route of administration to deliver the drug
• Understand how the blood (or plasma) levels relate to efficacy (PK-PD) or toxicity (TK-TD) in order to select safe doses
• Decide on the frequency and duration of dosing in order to sustain drug at target for disease modification

• Predict Human pharmacokinetics
• What is pharmacokinetics (PK)?
• Why do we need PK?
• How do we do PK?
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PERFORMING A PK STUDY

DOSE

COLLECT SAMPLES (BLOOD, URINE, BILE, FECES) AT VARIOUS TIMEPOINTS

ANALYZE FOR DRUG/METABOLITES

PK DATA ANALYSIS
Cassette dosing can enable rapid SAR on clearance (CLp)!

Confirm cassette PK parameters with discrete dosing design
• What is pharmacokinetics (PK)?
• Why do we need PK?
• How do we do PK?
• **How is the data generated?**
• How do we do PK analysis?
Plasma Concentration vs Time Curves

Electrospray ionization MS revolutionized drug discovery! (plasma, urine, bile analysis)
What happens to a drug when taken orally (or iv, ip, sc)?

- Pharmacokinetics (PK) – the mathematics of the time course of Absorption, Distribution, Metabolism, and Excretion of drugs in the body.

- A favorable PK profile is vital to the therapeutic success of a drug.

- Drug must be able to reach its intended target.
Plasma Concentration vs Time Curves

Relationship between the plasma concentration–time curve obtained following a single extravascular dose of a drug and parameters associated with the therapeutic or pharmacological response.
FEATURES OF AN IV BOLUS PK CURVE

- **Drug experiences only distribution and elimination**
- **$C_{\text{max}}$** is not observed, it is calculated (by extrapolation) as the Conc at $t=0$
- The terminal phase half-life is the true elimination half life of the drug

**Equations**

- Mono-exponential: $C = C_0 \cdot e^{-kt}$
- Bi-exponential: $C = Ae^{-\alpha t} + Be^{-\beta t}$

**Graphs**

- **Terminal Phase**
  - Slope of the line = $k_e$ or $\beta$ = Elimination rate constant
  - $t_{1/2} = \frac{0.693}{k_e}$ or $0.693/\beta$

- **Pre-terminal Phase**
  - Distribution and Elimination

- **$C_{\text{max}}$**
  - Concentration at time = 0
  - $C_{\text{max}}$ not observed only calculated
FEATURES OF AN ORAL PK CURVE

- Usually Absorption is faster than elimination i.e. $K_a \gg K_e$
- As $K_a$ approaches $K_e$; $C_{max} \downarrow$ and $T_{max} \uparrow$ (for same Dose)

$K_a$ and $K_e$ are rate constants related to absorption and elimination respectively.

- Absorption is not rate limited
- Terminal phases are parallel
- Same Dose = Same AUC
Intravenous

Advantages:

• No absorption stage - immediate effect without delay

• Guaranteed 100% bioavailability - no variation between patients

Disadvantages:

• Person administering dose needs careful training

• Sterility essential

• Possible extravasation
Extravascular administration

Any route other than i.v.

- An absorption stage will be involved
- Bioavailability may not be 100%

Principalally oral but also intramuscular, subcutaneous etc
Blood drainage from G.I.T.

- Mouth
- Stomach
- Small intestine
- Large intestine
- Rectum

General circulation

Liver
Extravascular administration

Almost pure elimination

Absorp $= \text{Elim}$

Absorp $<< \text{Elim}$

Absorp $>> \text{Elim}$

Almost pure elimination
Oral

Advantages:

• Simplicity

Disadvantages:

• Low and unpredictable bioavailability for some drugs

• Rate of absorption - slow and unpredictable
  
  - Release from the tablet/capsule etc
  
  - Gastric emptying
Rate of absorption of drug may be limited either by rate of release from injection vehicle or the ability of the blood to carry the drug away.
Subcutaneous

Drug has to carried away from the injection site by blood or lymph. Both blood and lymph flows to the subcutaneous tissue are poor. Release therefore rather slow.

If slow release is wanted, probably better to use a slow release formulation (Oil or plastic).

Can be painful.
Topical

With topical application, intention is generally to achieve a local effect. However, in many cases, drug will be absorbed into the general circulation.

Examples:

• Inhaled steroids intended to act in the lungs, but are absorbed and can cause some adrenal suppression.

• Beta-blockers in eye-drops reach measurable concentrations in blood.
**IV vs. ORAL**

**IV**
- Drug can be accurately dosed with a high level of control
- No absorption and hence bioavailability, first pass etc. is not an issue
- Rapid availability of drug for efficacy. No delays
- Not practical
- Patient compliance = 0
- Requires trained medical personnel for administration
- Extravasation can cause severe local toxicity
- Once administered there is no recall

**ORAL**
- Convenient and Safe
- Large surface area for absorption
- Food and varying pH at different parts of the GI tract can facilitate absorption
- Less abrupt change of drug concentrations than with parenteral administration
- First pass metabolism by the liver
- Relatively slow onset of action
- Absorption can be rate-limited by large particle size and poor dissolution
- Sensitivity to acid or digestive enzymes
- Presence, type and temperature of food
- Gastric emptying time
- Intestinal motility
• What is pharmacokinetics (PK)?
• Why do we need PK?
• How do we do PK?
• What do the data look like?
• **How do we do PK analysis?**
PK DATA ANALYSIS

NON COMPARTMENTAL METHODS
(STATISTICAL MOMENTS)

COMPARTMENTAL ANALYSIS
(SUM OF EXPONENTIALS MODELING)
Determine shape of profile on semi-log scale

Evaluate the # of kinetic phases observed

Select a 1 or 2 or 3 exponential equation based on # of kinetic phases

Best fit the observed data to the model using statistical ‘goodness of fit’ criterion.

WinNonLin 5.3

OBTAIN PK PARAMETERS
COMPARTMENTAL MODELING
(TWO COMPARTMENTS)

Rapid distribution to highly perfused tissues characterized by ‘alpha’ phase with a distributive half-life \( t_{1/2}(\alpha) \)

After distribution equilibrium, terminal ‘beta’ phase representing elimination with an elimination half-life \( t_{1/2}(\beta) \)

\[
C = A e^{-\alpha t} + B e^{-\beta t}
\]
NON-COMPARTMENTAL ANALYSIS (rapid approach)

- Determine shape of profile on semi-log scale
- Perform linear regression on terminal linear (hopefully) phase
- Determine slope of the line. This is the elimination rate constant ‘Ke’
- Estimate AUC (Area Under the Curve) and AUMC (Area Under the first Moment Curve) by trapezoidal rule

WinNonLin 5.3
AREA UNDER THE CURVE

TRAPEZOIDAL RULE

\[ \text{AUC}_{\text{last}} = \sum \frac{C_n + C_{n-1}}{2} \cdot t_n - t_{n-1} \]

\[ \text{AUC}_\infty = \text{AUC}_{\text{last}} + \frac{C_{\text{last}}}{K_e} \]

\[ \text{AUC}_\infty = \int_0^\infty C \cdot dt = \sum_1^n \frac{C_i - 1}{\lambda_i} \]

➤ Measure of systemic exposure
➤ AUC proportional to Dose = Linear pharmacokinetics
VOLUME(S) OF DISTRIBUTION (V)

\[ V_{\text{app}} = \text{Amount of drug in body at equilibrium} \]
\[ \text{Drug Conc. in plasma (blood)} \]

- No physiological significance but its magnitude gives a general idea of extent of distribution in the body.
- Monoexponential kinetics are explained by a single volume \((V_d)\).
- Multi-exponential kinetics yield an instantaneous volume \((V_c)\), a terminal volume \((V_\beta)\) and a steady state volume \((V_{ss})\).
**VOLUMES OF DISTRIBUTION:**

Examples

<table>
<thead>
<tr>
<th>Drug</th>
<th>Vd, (human, L/kg)</th>
<th>Distribution Characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Warfarin</td>
<td>0.1</td>
<td>Small vol. Mainly stays in plasma little in tissues.</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>0.23</td>
<td>Medium vol. Similar concs in plasma and tissues</td>
</tr>
<tr>
<td>Theophylline</td>
<td>0.5</td>
<td>Large vol. Mainly in tissues, little in plasma.</td>
</tr>
<tr>
<td>Cimetidine</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Digoxin</td>
<td>7.3</td>
<td></td>
</tr>
<tr>
<td>Mianserin</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>Quinacrine</td>
<td>714</td>
<td></td>
</tr>
</tbody>
</table>
CLEARANCE (Cl)

Defined as the volume of blood that is completely cleared of the drug/unit time when it passes through a clearing organ.

Cl = \frac{\text{Dose}}{\text{AUC}_\infty}

For linear pharmacokinetics:- Cl is constant as a function of dose
Cl is additive i.e. Cl_{tot} = Cl_{hepatic} + Cl_{extra-hepatic}
Cl is best calculated from IV data (f_{bioavail} is not a confounding factor)
Cl cannot exceed the cardiac output of the animal
Clearance Mechanisms
Top 200 Drugs Prescribed in US

Route of Clearance of Top 200 Prescribed Drugs

Metabolite assessment in vitro & in vivo aids in identifying enzymes involved in clearance

More recent analyses consistent with these results

J. A. Williams et al. 2004 Drug Metab. Dispos. 32, 1201-1208
Origin of the Term ‘Cytochrome P450’

- Heme iron in P450 is usually in the ferric (Fe$^{+3}$) state
- When reduced to the ferrous state (Fe$^{+2}$), P450 can bind ligands (O₂, CO).
- The complex between ferrous P450 and CO absorbs light maximally at 450 nm
- Cytochrome P450 derives its name from this peak absorbance

Omura and Sato (1962) *J. Biol. Chem.* 237, 1375-6
### P450s & Substrates

#### SUBSTRATES

<table>
<thead>
<tr>
<th>1A2</th>
<th>1A2</th>
<th>2B6</th>
<th>2C8</th>
<th>2C9</th>
<th>2C9</th>
<th>2C19</th>
<th>2C19</th>
<th>2D6</th>
<th>2E1</th>
<th>2E1</th>
<th>3A4,5,7</th>
</tr>
</thead>
<tbody>
<tr>
<td>clozapine</td>
<td>cyclobenzaprine</td>
<td>duloxetine</td>
<td>fluvoxamine</td>
<td>haloperidol</td>
<td>imipramine</td>
<td>mexiletine</td>
<td>nabumetone</td>
<td>naproxen</td>
<td>olanzapine</td>
<td>risperidone</td>
<td>theophylline</td>
</tr>
<tr>
<td>bupropion$^1$</td>
<td>cyclophosphamide</td>
<td>efavirenz$^1$</td>
<td>efavirenz$^1$</td>
<td>efavirenz$^1$</td>
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</tr>
<tr>
<td>paclitaxel</td>
<td>torsemide</td>
<td>amodiaquine$^2$</td>
<td>cerivastatin</td>
<td>repaglinide</td>
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</tr>
</tbody>
</table>

#### GENETICS

| 1A2          | 2B6          | 2C8          | 2C9          | 2C9          | 2C19         | 2C19         | 2D6          | 2E1          | 3A4,5,7      |
|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|
| Chr          | Chr          | Chr          | Chr          | Chr          | Chr          | Chr          | Chr          | Chr          | Chr          | Chr          | Chr          |

#### Polymorphisms in Humans

Polymorphisms in humans can impact PK of drugs

PM = poor metabolizer
EM = extensive metabolizer
IM = intermediate metabolizer

[http://medicine.iupui.edu/clinpharm/ddis/ClinicalTable.asp](http://medicine.iupui.edu/clinpharm/ddis/ClinicalTable.asp)
Aliphatic Hydroxylation

Tolbutamide

CYP2C9

Lauric acid

CYP4A

ω -1 hydroxylation

ω hydroxylation

Testosterone

CYP3A4
Aromatic Hydroxylation

- Chlorzoxazole
- Coumarin
- (S)-Mephenytoin
Mammalian Conjugation & Potential for sustained exposure
Enterohepatic recirculation potential for drug-drug interaction and impact on PK (e.g., oral contraceptives and antibacterials)
HALF-LIFE \((t_{1/2})\)

The time it takes for the amount or concentration to fall to \(\frac{1}{2}\) its original value

- For 1\(^{\text{st}}\) order processes: \(t_{1/2} = 0.693/k\)

PK Half life is a dependent parameter and depends on \(\text{Cl}\) and \(V\)

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

- Half life helps to determine time to steady state in an infusion or repeat dosing
- Helps to select the dosing interval in multiple dosing
Contribution Factors to Dosing for orally dosed small molecules

- Volume of Distribution
- Clearance
- Absorption

- Half-life
- Bioavailability

- Duration of exposure needed
- PK/PD
- Efficacious Concentration

- HOW OFTEN
- HOW MUCH

Reflection of Oral Clearance (CL/F)

Adapted from Obach RS, Current Opinion in Drug Discovery and Development 2001 4(1):36-44.
Multiple Dosing

TOXICOKINETICS

If $\frac{AUC_{DAY \ n}}{AUC_{DAY \ 1}} = 1$ then
NO ACCUMULATION OR AT SS

If $\frac{AUC_{DAY \ n}}{AUC_{DAY \ 1}} < 1$ then
INDUCTION (adaptive response – P450 levels increased – increased metabolism)

If $\frac{AUC_{DAY \ n}}{AUC_{DAY \ 1}} > 1$ then
ACCUMULATION/CLp SATURATED

5 half-lifes to steady state
Test a pharmacology hypothesis

...by improving the exposure of your test article with a P450 inactivator
Resolving exposure issues in rodent POC (leveraging a pan-P450 inactivator)

1-aminobenzotriazole (ABT)

PO410155 PO 10 mg/kg

mGluR4 positive allosteric modulator

Cytochrome P450: Structure, Mechanism and Biochemistry, 3rd Ed. P.O. deMontellano
Contributing Factors to Dosing for orally dosed small molecules

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