**Pharmacokinetics**

**Pop quiz**

Which of these compounds has higher plasma clearance?

Which of these compounds is eliminated more rapidly?

Which has a longer half-life?

Which is more distributed to tissues?

Which has a higher Volume of Distribution ($V_d$)?

Is it plausible that either could be solely eliminated renally?

Is it plausible that either could be solely metabolized hepatically?

**MOST IMPORTANT: What are the implications of respective PK for these compounds as drug leads?**

**Which would be a better drug?**

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**Today's learning goals**

- Learn the physiological meaning behind each of the standard PK measurements
- Learn how a “goal”/“target-product profile” can be set for each of these measurements, how that relates to PK/PD, (and why the goals are different program-by-program)
- Be able to quickly look at a PK curve and assign qualitative/relative values to each PK term
- Learn general trends for how chemical structure affects PK

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**Not covered**

- Rigorous quantitative kinetic treatment of PK (no math here, folks!)
- Exhaustive listing of bioisosteric approaches to PK improvement
- Deep dives into mechanisms of metabolism (no CYP or AO protein structures will be discussed)
- Any discussions of toxicology

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Adapted from Randy Miller’s “Pharmacokinetics,” Residential School on Medicinal Chemistry, 2017.
**Pharmacokinetics**

**Drug kinetic lifecycle — a simplified picture**

- Drug (dose IV) enters the central compartment.
  - Metabolism/excretion
  - Hepatic clearance
  - Renal clearance
  - Distribution

- Drug (dose PO) enters the GI tract.
  - Adsorption
  - %F
  - Distribution
  - V_d

**Central compartment**
- C_u,plasma
- C_bound,plasma (PPB)

**Peripheral compartments**
- C_u,tissue
- C_bound,tissue

**Single compartment vs. multicompartment**

If drug has low V_d and target is central, sometimes single compartment model is acceptable.

Two PK profiles, after IV dosing:
- Single-compartment model appropriate:
- Two-compartment model required:
  - "distribution phase" (alpha phase)
  - "terminal phase" (beta phase)

**What we measure vs. what impacts pharmacology and kinetics**

- Typically, total plasma concentration measured *in vivo*
- Must correct for PPB! (But more PPB is NOT a bad thing, see later slides)
- Inherent assumption that C_u,targetTissue tracks with C_u,plasma (Sometimes false)

**FREE DRUG HYPOTHESIS:** "Only unbound drug in the compartment of interest mediates pharmacological events. That is, clinical effect tracks with C_u."**

**Starting point: “Necessary coverage” (unbound, trough)**

- Standard multi-dose, QD PO, PK curve:
  - t_{max}
  - C_{max}
  - V_d affects alpha phase
  - V_d, CI affect beta phase
  - %F and adsorption kinetics affect absorb phase, t_{max}, C_{max}

**Fundamental goal of PK for med. chemists:**
- Understand how each term affects this curve shape
- Understand how to optimize compound structure to impact each term

**Term cheat-sheet**

- PO: per os; dosed orally
- IV: intravenous
- %F: Percent oral bioavailability
- PPB: Protein-plasma binding
- Cl: Clearance
- t_{1/2}: half-life
- QD: quaque die; once-a-day (similarly, BID = twice-a-day)
- C_{max}: maximum concentration
- t_{max}: time of maximum concentration

**Terms in blue are measurable/calculable observables**

**Cartoons reproduced from:**
Pharmacokinetics

Oral bioavailability (often called %F; F; BA, ‘fraction available’)

%F = AUC_{PO} / AUC_{IV} * (dose_{IV} / dose_{PO})

What it tells us: Relative total exposure of drug when dosed PO vs. IV
How it’s measured: Dose PO and IV. Measure each’s AUC. Dose-corrected AUC ratio is %F.
What it affects: The dose needed.
Optimal value: Higher is better. >20% is good, >5% often workable.
Common pitfalls in understanding: Note that %F is a relative kinetic measure (absorption relative to all other PK processes), not an absolute kinetic measure of absorption. Thus, C_{max} and t_{max} may or may not track with %F.

Case study to illustrate above pitfall: Ketoprofen (NSAID) PO dosing in fasted or fed state:

Male volunteers, age = 22-35, n = 12. Fed state is “large breakfast 1 hour before administration.”

Identical doses, identical AUC’s, thus, identical %F
C_{max,fasted} = 2.8 vs. 7.6 h for fed (63% decrease)
C_{max,fasted} = 12.1 vs. 8.0 for fed (34% increase)

Upshot: %F cannot wholly predict C_{max} or t_{max}!

Simplified biological picture of absorption:

To be absorbed, a drug must:
- Dissolve in water
- Survive the stomach
- Permeate the gut wall membrane
- Survive first-pass metabolism

Key factors that affect oral bioavailability

Water solubility: More is better for dissolution. Tracks inversely with lipophilicity.
Acid stability: Stomach pH is ~2.5
Permeability: Drug must pass through lipophilic membrane. Generally tracks with lipophilicity.
Metabolic stability: Drug must either survive first-pass metabolism, or have sacrificial pathway for first metabolism (prodrug)

How to tell where your problem is?
Isolate variables. If Cl < Q_{b} (see Clearance section), then low ER and likely not metabolism issue. Probe membrane permeability directly, with...

Caco-2, MDCK and PAMPA: Assays for permeability

Approach: Grow cells (Caco-2 or MDCK) on a filter transwell insert (aka, a frit). Resultant film resembles human intestinal mucosa. Put compound on one side, measure kinetics of diffusion. More sophisticated experiments measure bidirectional diffusion, and doped efflux inhibitors can determine active transport contribution. PAMPA is synthetic equivalent.


Meta-analysis across hundreds of NSAID studies: %F doesn’t change fed/fasted

Meta-analysis across hundreds of NSAID studies: t_{max} consistently decreases when fasted

Analgesic effect correlates with C_{max} not AUC!

Upshot: fasted dosing slightly increases gastric ADEs, but dramatically improves analgesic efficacy

Terms in blue are measurable/calculable observables

Madin-Darby Canine Kidney cells
Heterogeneous human epithelial colorectal adenocarcinoma cells

Solomon H. Reisberg

Pharmacokinetics

Case studies on improving %F
Via improving aqueous solubility

Increase $Sp^3$ fraction

Strategies: Sure, just making the molecule more polar helps. But that causes other issues. Try to increase solubility without necessitating decreased lipophilicity...

Disrupt molecular symmetry


Use heteroatoms to twist fused bicycles

Ciprofloxacin, solubility: 0.08 mg/mL

Cipro analog, 0.25 mg/mL (also 2X potency)

Via improving stomach pH stability

Azithromycin (Pfizer), $X_1 = N; X_2 = CH_2; R = H$

Clarithromycin (Taisho), $X_1 = CH; X_2 = CO; R = Me$

gastric acid stable; greatly improved %F!

%F = 38 for azithromycin;

%F = 50 for clarithromycin;

%F = 0 for unformulated erythromycin

Upshot: Rational understanding of metabolites can impact design for more than just clearance

Outdated dogma is Lipinski’s Rule of 5, which says achieve permeability by

- LogP 0 to 5
- MW < 500
- <10 H-bond acceptors
- <10 rotatable bonds
- <140 PSA

These requirements are impossible within the complexity of modern drugs and their "undruggable" targets. Nonetheless, good starting point.

However, they lay the strategy: Reduce conformational flexibility; increase lipophilicity.

Mask HBD’s with intramolecular acceptors

PGP efflux liability

Diminish acidity/basicity

Better potency, lost permeability pKa = 9.7

Permeable and potent pKa = 8.8
Scheme 4

**Reactions:***
- **27-31, 33, 35**
  - A = F
  - 1. NH$_2$NH$_2$
  - 2. O$_2$/NaOH

- **36**, 47-91%

- **35, A = CH$_3$**

- **37**, 80-96%
  - 1. TFA
  - 2. POCl$_3$/DMF
  - 3. HCl/EtOH

- **38, 17-66%**
  - 1. LiAlH$_4$
  - 2. Swern
  - 3. diethyl malonate

- **39**
  - Dowtherm A

- **40**, 26-73%
  - R$_2$R$_3$NH, 9

- **41**
  - 1. LiOH
  - 2. HCl

- **42-49**
  - 46-83% in three steps

**Compounds:***
- **42**: X = Cl, Y = H, R$_4$ = Cyclopropyl
- **43**: X = F, Y = H, R$_4$ = Cyclopropyl
- **44**: X = H, Y = H, R$_4$ = Cyclopropyl
- **45**: X = Me, Y = H, R$_4$ = Cyclopropyl
- **46**: X = Et, Y = H, R$_4$ = Cyclopropyl
- **47**: X = OMe, Y = H, R$_4$ = Cyclopropyl
- **48**: X = Me, Y = H, R$_4$ = Et
- **49**: X = H, Y = Me, R$_4$ = Cyclopropyl
Via improving first pass metabolism

In general, approaches for first-pass metabolism are same as improving clearance; see later slides.

Metabolic stabilization approaches unique to first-pass:

**Pro-drugging**

Esters, phosphates, aminals, carbamates, peptide conjugation, commonplace.

More interesting:

- Designed-in metabolites
- Designed-in designed-in metabolites (e.g., 11-hydroxy THC)

**On %F and abuse prevention**

MOR agonist, %F = 99

*Tilidine, potent MOR agonist, %F = 99 (first-pass demethylates)*

Formulated together: If taken orally (as prescribed), naloxone has no effect. If taken IV (off label), naloxone reduces analgesic efficacy.

**Volume of distribution (Vd)**

1 L of water
Dissolve 1 g of NaCl
measure concentration; 1 g/L
apparent volume: 1 L

1 L of 1:1 water / DCM
Dissolve 1 g of NaCl
measure concentration; 2 g/L
apparent volume: 2 L

**What it tells us:** Relative distribution of drug to plasma and tissue.

**How it’s measured:** Dose IV. Immediately after distribution, measure plasma C.

**What it affects:** The shape of the PK curve. Namely, higher volumes lead to higher t₁/₂.

**Note:** t₁/₂ = 0.693 * Vd / Cl

**Optimal value:** Completely program dependent. Intracellular targets need higher volumes. A high Vd can be used to counteract a high clearance. A low Vd will increase Cmax and increase the proportion of the AUC at high levels of plasma exposure.

**Case studies on improving %F (cont.)**

**Via improving first pass metabolism**

**Case study to illustrate above pitfall:**

**Volumes track very well across organisms**

For this reason are often reported as L/kg (bodyweight normalized). If not normalized, here are physiological volumes for reference:

<table>
<thead>
<tr>
<th>Animal</th>
<th>Plasma</th>
<th>Extracellular fluid</th>
<th>Total Body water</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse</td>
<td>70 mL/kg</td>
<td>3.0 L (0.04 L/kg)</td>
<td>6.0 L (0.6 L/kg)</td>
</tr>
<tr>
<td>Rat</td>
<td>2.5 kg (0.04 kg)</td>
<td>1.1 L (0.04 L/kg)</td>
<td>1.5 L (0.2 L/kg)</td>
</tr>
<tr>
<td>Mouse</td>
<td>250 g (0.05 g)</td>
<td>0.11 L (0.04 L/kg)</td>
<td>1.1 L (0.5 L/kg)</td>
</tr>
<tr>
<td>Rat</td>
<td>10 g (0.05 g)</td>
<td>0.01 L (0.04 L/kg)</td>
<td>0.05 L (0.1 L/kg)</td>
</tr>
</tbody>
</table>

**Key factors that affect Vd**


**Low volume drugs** are either highly PPB, or too polar to bind to tissues. Vd < 0.4 L/kg

**High volume drugs** are highly bound to tissues. Usually very lipophilic. Vd > 1 L/kg

**Ionization is most important feature.** Vd trend, all else equal:

- Basic (cationic) > neutral > zwitterionic > acidic (anionic)

**Why?**

- In tissues, cations get “stuck” to anionic phospholipid heads - low fubound,tissue
- Bases are held within cells by pH gradient (pH 7.2 intracellular vs. 7.4)
- Acids bind more tightly to HSA, and thus have lower fubound,plasma

**Vd tracks secondarily with lipophilicity.**
Case studies on manipulating $V_d$

Disclaimer: $V_d$ is very rarely optimized directly. Volumes are less critical than potency, %F, Cl, and the changes necessary to improve $V_d$ often negatively impact the other parameters.

These cases studies are at best exceptions to the rule, and at worst, lucky coincidences.

via ionization control

Inconsistent animal models with highly ionized compounds

Pfizer Histamine H3 antagonists, Drug Metab. Dispos., 2009, 37, 1864

upshot: adding basicity can dramatically improve $V_d$ (and thus $t_{1/2}$);
be wary impact on other parameters. Beware cardiac toxicity.

Via lipophilicity modification

In general, $V_d$ tracks with lipophilicity. However, $Cl$ also tracks with lipophilicity. For that reason, non-strategic increases to LogD will usually cancel themselves out in impact to $t_{1/2}$.

beta blockers

Amlodipine, $V_d = 21$ L/kg
dosed QD

Calcium ion channel blockers. Result: Amlodipine is 5th most prescribed med in US; nifedipine is 154th.


branerutinib, $V_d = 0.5$ L/kg

CaR inhibitors imitate hypocalcemia. If short $t_{1/2}$, induce catabolism and bone loss.


dangerous game to play:

Clinical trials aborted due to low plasma exposure.


Upshot: increased volume, increased half-life, for better or worse. Adding lipophilicity or adding basicity to increase volume comes at a strategic cost.

Low lipophilicity, low volume, low solubility:

Can “mimic” a high-volume PK profile.

BMS HIV attachment inhibitor $V_d = 0.05$ L/kg; $t_{1/2} = 1$ h (cyano)

However, with prodrug:

$R = CI$, (rat) $V_d = 11$ L/kg, $t_{1/2} = 2.1$ h, causes osteoporosis!

$R$ = H, $V_d = 0.82; t_{1/2} = 2.6$

half-life trends rationalizable with $Cl$ (glucuronidation), but 2X volume DECREASE with lipophilic methyl is surprising.


upshot: adding basicity can dramatically improve $V_d$ (and thus $t_{1/2}$);
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via ionization control

inconsistent animal models with highly ionized compounds

Ensemble: $V_d = 12$ L/kg, $t_{1/2} = 18$ h
permeability regained

Pfizer NaV1.7 inhibitor (pain)

PKa = 6.3, 7.5 (zwitterionic)

$V_d$ (rat) = 1.3 L/kg

$V_d$ (dog) = 0.2 L/kg

Result? Cautionary microdosing in man before further clinical investigation.

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In this case, lower $t_{1/2}$ is desired to clear before ADEs


dangerous game to play:

Clinical trials aborted due to low plasma exposure.


Upshot: increased volume, increased half-life, for better or worse. Adding lipophilicity or adding basicity to increase volume comes at a strategic cost.
Scheme 3. Apparent Manufacturing Route to Ibrutinib, Final Steps

3 + 12-R \rightarrow 13

\[ \text{Et}_3\text{N, H}_2\text{O, EtOH} \]

\[ \text{EtOH, HOAc} \]

\[ \text{NH, H, NH}_2 \]

\[ \text{120 °C} \]

\[ \text{Pd(OH)}_2/C \]

\[ \text{H}_2, \text{MeOH} \]
Pharmacokinetics

What it tells us: Rate constant (not rate): apparent volume of blood from which drug is “cleared” (metabolized to other compounds + excreted to urine + excreted to feces) per unit time

How it’s measured:
- in vitro: Hapatocyte and microsome assays determine in vitro $t_{1/2}$
- in vivo: Cl = dose / AUC

Units are mL/min (absolute), or mL/min/kg (normalized to bodyweight)

What it affects: Lower clearances lead to higher $t_{1/2}$. Lower clearances lead to higher AUC. Thus, changing clearance has impact on dose AND dosing interval.

Clearance

Optimal value of Cl:

Effect of PPB on Cl (UPSHOT, PPB is NOT IMPORTANT)

Hepatocyte and microsome assays work: Compound is incubated with hepatocytes (whole cell liver tissue) or microsomes (centrifuged supernatant of hepatocytes, which enriches in CYP). Measure parent compound conc (LC/MS/MS) vs. time.

Key note: If hepatocyte clearance is dramatically higher than microsome, it is likely that a non-CYP pathway is primary.

Cl_{int} prediction is notoriously species-specific, and IV/IV correlations are often bad:

- 10-fold change in PPB does NOT inherently change C_{int}
- 10-fold change in PPB has direct reduction on Cl (for low-mid ER drugs)

Higher PPB slows clearance! But this effect is cleanly canceled out by lower unbound exposure for on-target effect.

Term cheat-sheet

DDI: drug-drug interaction

IV/IV: in vitro/in vivo

Reproduced from: Tozer and Rowland, "Introduction to Pharmacokinetics and Pharmacodynamics"
**Pharmacokinetics**

**Case studies on improving clearance**

**Reducing CYP-mediated clearance**

**Strategy 1: Lower lipophilicity**

![Chemical structure 1](image1)

HLM Clint (mL/min/kg)

<table>
<thead>
<tr>
<th>R = H</th>
<th>R = F</th>
</tr>
</thead>
<tbody>
<tr>
<td>176</td>
<td>156</td>
</tr>
<tr>
<td>70</td>
<td>29</td>
</tr>
</tbody>
</table>


**Strategy 2: Rationally block metabolic hotspots**

![Chemical structure 2](image2)


**Strategy 3: Stick a fluorine on it**

Strategy 4: KIE

Other common strategies:
- Add EWGs to aromatics to increase redox potential
- Block alpha-positions to carbonyls
- Replace esters with amides
- Avoid phenols and anilines (Ames and glucuronidation)

---

*Several of these examples taken from excellent review, See: Med. Chem. Comm., 2013, 4, 631.*
Pharmacokinetics

Case studies on improving clearance (cont.)

Reducing AO-mediated clearance

Issues with Aldehyde Oxidase:
- Clearance tracks inversely with lipophilicity (vs. directly for CYPs)
- AO is absent in HLM, so liabilities may be missed
- AO is underexpressed in the dog, so liabilities may be missed
- AO is widely-distributed, so a high Vd may not buffer t1/2
- More generally, IV/IV disconnects are rampant

Metabolism is primarily oxidation of imine and e-deficient imine-like heterocycles

For an excellent review on AO, see: J. Med. Chem., 2010, 53, 8441.

Renal clearance

- Note that renal clearance is generally small for drug-like small molecules
- This is because Clrenal tracks inversely with lipophilicity, and so most drugs have low ERrenal
- And also because Qrenal << Qg
- Clrenal can show pseudo-0th-order kinetics because of resorption effects:
  For the rare exception molecules that are renally metabolized, it is often via active transport (OAT or OCT)
  Example, OAT excretion for methotrexate

Strategies to mitigate AO metabolism

Increasing lipophilicity generally helps, but has other issues (CYP). Other approaches...

Enrich electronics of ring

KIE/deuteration approaches don't work with AO (C-H abstraction is not RDS) J. Med. Chem., 2010, 53, 8441.

Block site

AO unstable

AO stable


AO unstable

AO stable


AO unstable (potency lost)

AO stable (potency regained)

Dirty secret:
Everything tracks with lipophilicity!
(often including potency)
(but in different directions)

Assuming all of AUC is efficacious!

Renal clearance

Methotrexate / NSAID issues

- Most NSAIDs are OAT inhibitors (but not transportees)

MeO

nnaproxen sodium (Aleve)

in combination with methotrexate, 50 to 140% increase to methotrexate t1/2!

Pharmacokinetics

Intestinal targeting via intentionally-low %F


Liver targeting via OATP transporters


Idea: Maintain low passive exposure (usually via low permeability)
- Optimize for active transport via OATP.
- Ideal properties:
  - acid or diacid
  - OATP1B1 or 1B3 substrate
  - low permeability
  - logD (pH 7.4) 0.5 to 2
  - high water solubility

For example, Atorvastatin (lipitor):

$R = \text{Me}_3$, systemic distribution, dose-limiting hypoglycemia

$\text{CO}_2\text{H}$, hepatoselectivity, clinical candidate.


For ingenious "trapping prodrg" strategy for liver targeting, see sofosbuvir story:
Expert Opin. Drug Discov., 2015, 10, 1363.

Conclusions
- Drug efficacy is driven (in non-linear ways) by exposure of unbound drug in tissues ($C_{\text{bound,tissue}} \times $ time)
- The interplay of absorption, distribution, and clearance drive dosing and dose interval
- Optimization of each parameter is rationally guided but highly empirical.

Special approaches for selective tissue targeting

Phagocyte-mediated tissue accumulation

Figure reproduced from: J. Cont. Rel., 2017, 259, 53.

Idea: Many phagocytes accumulate selectively in certain tissues, especially in disease state.
Can selective binding to phagocytes lead to "cargo-laden" specific tissue delivery?
Modest success has been achieved with IV siRNA and peptidic drugs, e.g., see Mol. Ther., 2010, 18, 993.


Block permeability is analogous strategy.

Liver targeting


Idea: Maintain low passive exposure (usually via low permeability)
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