H3D Foundation and Ersilia Present

Bringing data science and AI/ML tools to infectious disease research

Session 3: ADME/PK in drug discovery

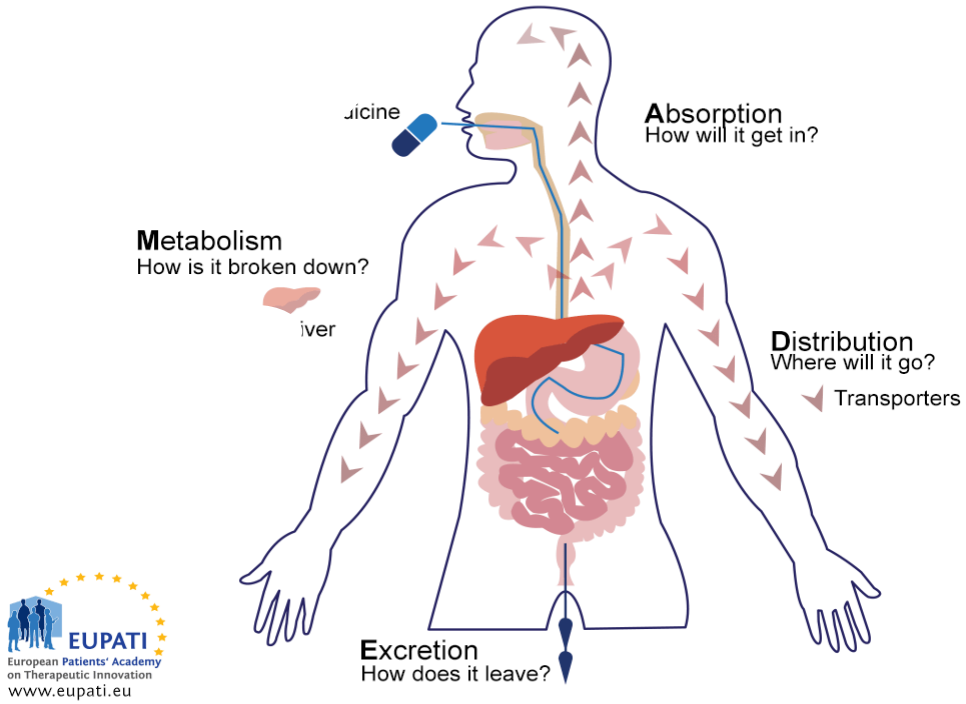
Event Sponsors

wellcome

CS&S Code for Science & Society

Introduction

- Why doesn't good *in vitro* potency translate to good *in vivo* potency for some compounds?
  - Biology reasons
  - ADME



- Can the drug get to the site of action at high enough levels and stay there for long enough to give the desired pharmacological effect?

Solubility and drug discovery

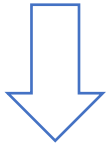
- **In vitro assays**
  - Needs to be soluble enough to give reliable *in vitro* activity data

Is my compound soluble enough for biological assays?

Measure solubility by adding DMSO stock to buffer (Kinetic solubility)

- **In vivo assays - Pharmacokinetics**
  - Needs to be soluble enough for absorption otherwise data will be poorly reproducible

Is my compound soluble enough for *in vivo* PK?



Measure solubility by adding buffer to solid compound (Thermodynamic solubility)

Solubility and drug discovery 2

Pharmacokinetics. In the first-in-human study, peak and total plasma exposures of MMV390048 generally appeared to increase with increasing doses (Fig. 2). However, considerable intersubject variability within each cohort was seen for all pharmacokinetic parameters (Table 3 and Fig. S2). The 20-mg cohort showed the least intersubject variability but a disproportionally high exposure with respect to other dose cohorts. The elimination half-life of MMV390048 (>149 h) was longer than was predicted from preclinical studies (90 h). The median time of maximum concentration (T_{max}) was 1 to 2.5 h after dosing for the fasted cohorts but was longer for the 40-mg fed cohort (4 h). Furthermore, administration of an FDA-prescribed high-fat breakfast (13) prior to dosing reduced intersubject variability for all pharmacokinetic parameters in comparison to the equivalent dose administered fasted, although variability was still moderate. Investigations were performed to assess whether any manufacturer or site factors explained or contributed to the high levels of interindividual variability observed. It was concluded that neither dosing nor packaging or preparation of MMV390048 were likely to be the source of variability.

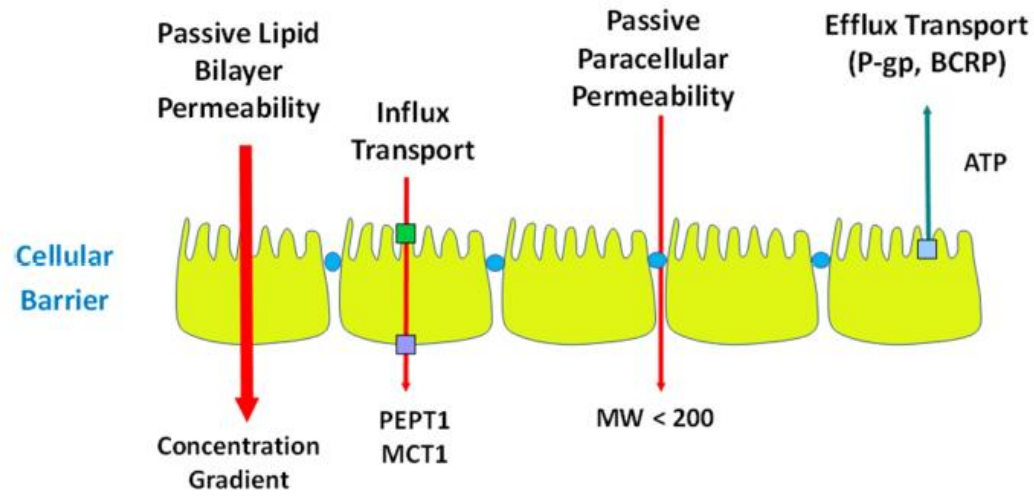
High intersubject variability was also observed in the pharmacokinetic parameters in the IBSM study (Table 3 and Fig. S3). MMV390048 plasma concentrations were only assayed in samples taken up to 192 h after MMV390048 dosing (Fig. 2). The remainder of the samples were not analyzed due to the significant variability observed and the sponsor's decision to suspend the study to reformulate the compound.

The first pharmacokinetic study of halofantrine was performed in 8 healthy male volunteers.[77] The aim of this investigation was to investigate the dose-proportionality of halofantrine and the main metabolite following single oral doses of halofantrine (as the tablet) 750, 1000 and 1250mg in healthy volunteers. Halofantrine was analysed by HPLC, but *N*-debutyl-halofantrine was not measured. Blood concentration-time data of halofantrine were fitted to a 2-compartment open pharmacokinetic model. The drug was absorbed slowly, reaching C_{max} values approximately 3 hours postdose. The terminal elimination half-life ($t_{1/2\beta}$) ranged from 1.3 to 6.6 days (table I). It was concluded that measures of bioavailability were not consistently dose-dependent, suggesting that absorption was incomplete or erratic.

Three years later, Wareham et al.[81] found no correlation between dose and C_{max} or area under the plasma concentration-time curve (AUC) over a dosage range of 250 to 1000mg (tablet formulation). However, the lowest C_{max} and AUC were found in the group of volunteers receiving halofantrine 250mg. At doses higher than 500mg, the further increases in C_{max} and AUC were small. It was clear from this study that there was large interindividual variability in C_{max} (table I) and the absorption of halofantrine was low and variable when the dose was higher than 500mg. The only

Permeability

- Permeability is a measure of the velocity of drug passage across membranes
- Drugs need to cross biological membranes to get to their site of action and interact with the respective targets
  - GI epithelilal cells
  - Blood capillaries
  - Hepatocyte membrane
  - Target cell membrane
- Passive permeability is the main route through which drugs get across cellular membranes



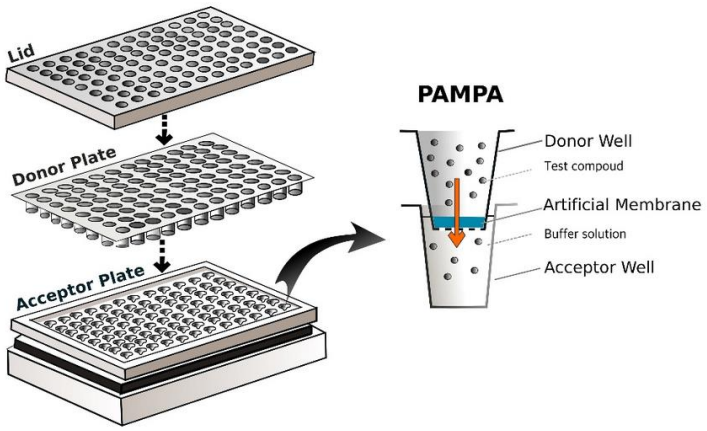
Measuring permeability

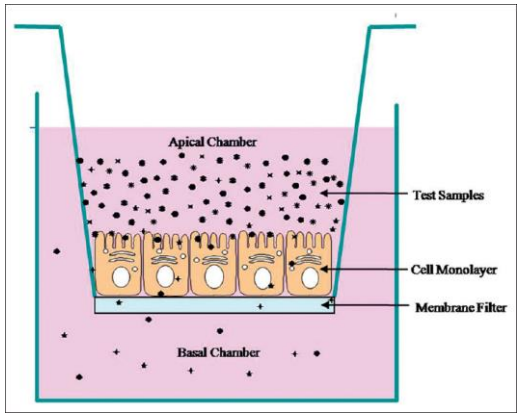
Parallel Artificial Membrane Permeability Assay (PAMPA)
Immobilized Artificial Membrane (IAM) HPLC

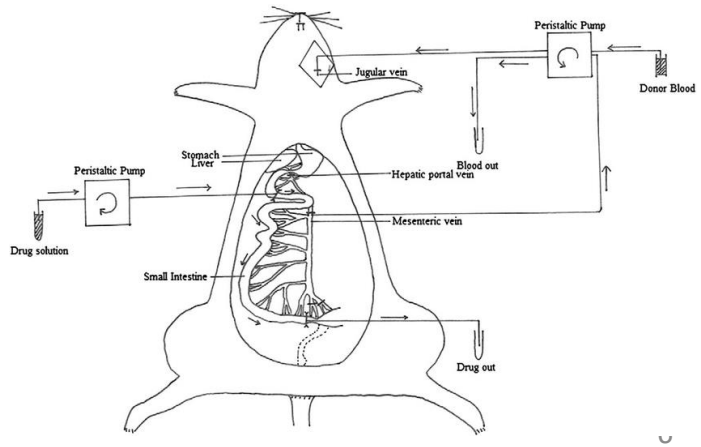
Caco-2
MDCK
HEK293
(wildtype and transporter knock out)

In situ perfusion
Hepatic portal vein cannulation

Increasing system complexity and cost
Reducing throughput







Solubility, Permeability and Dose

MAD = Solubility x Ka x SIWV x SITT

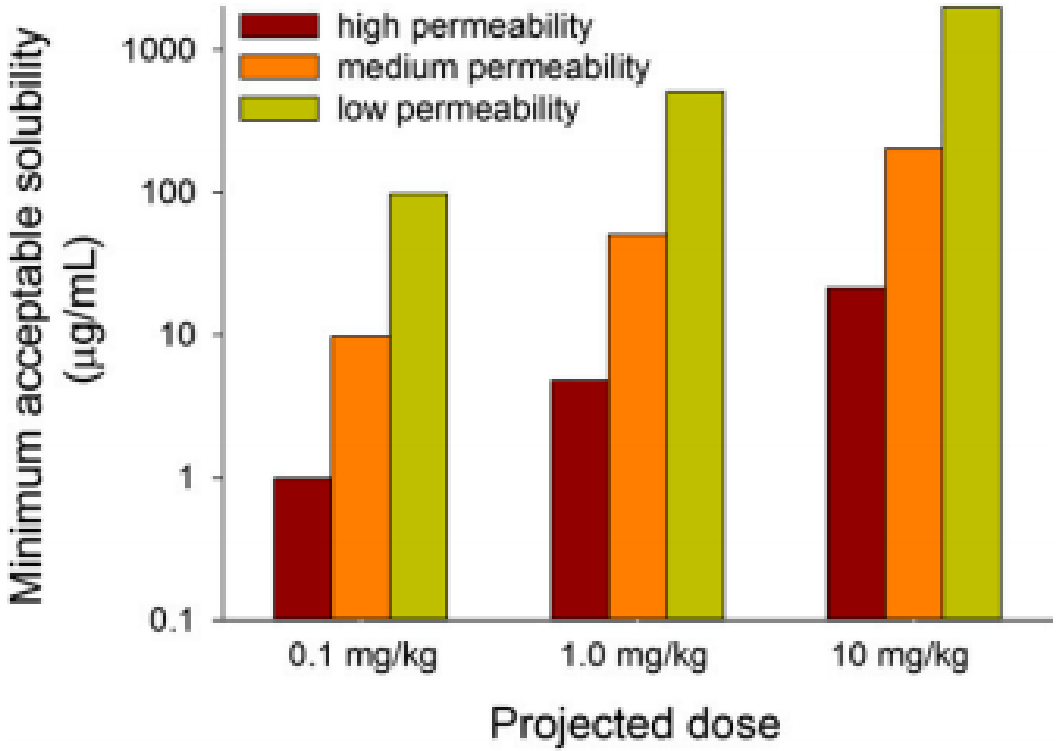
Solubility in µg/ml

Intestinal absorption rate constant (**permeability**)

Small intestine water volume

Small intestine transit time

- How soluble and permeable do malaria drugs (approx. 1mg/kg) and TB drugs (approx. 10mg/kg) need to be in order to avoid PK problems in the clinic?



Lipinski 2000 J Pharm Tox Methods 44, 235 – 249
Williams et al **2013** Pharmacol Rev 315 - 499

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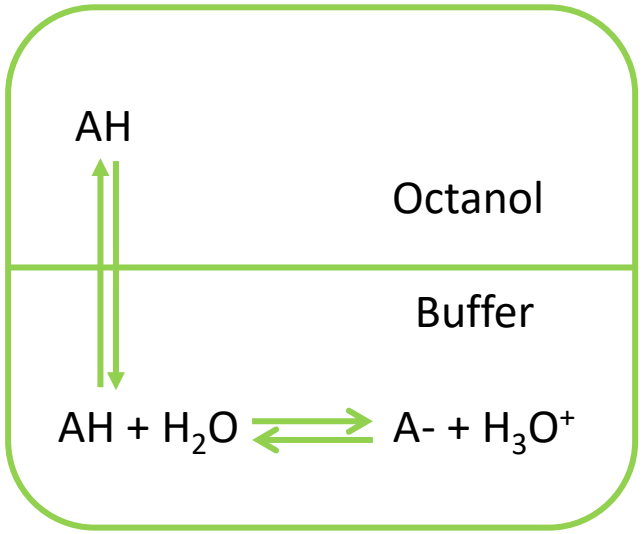
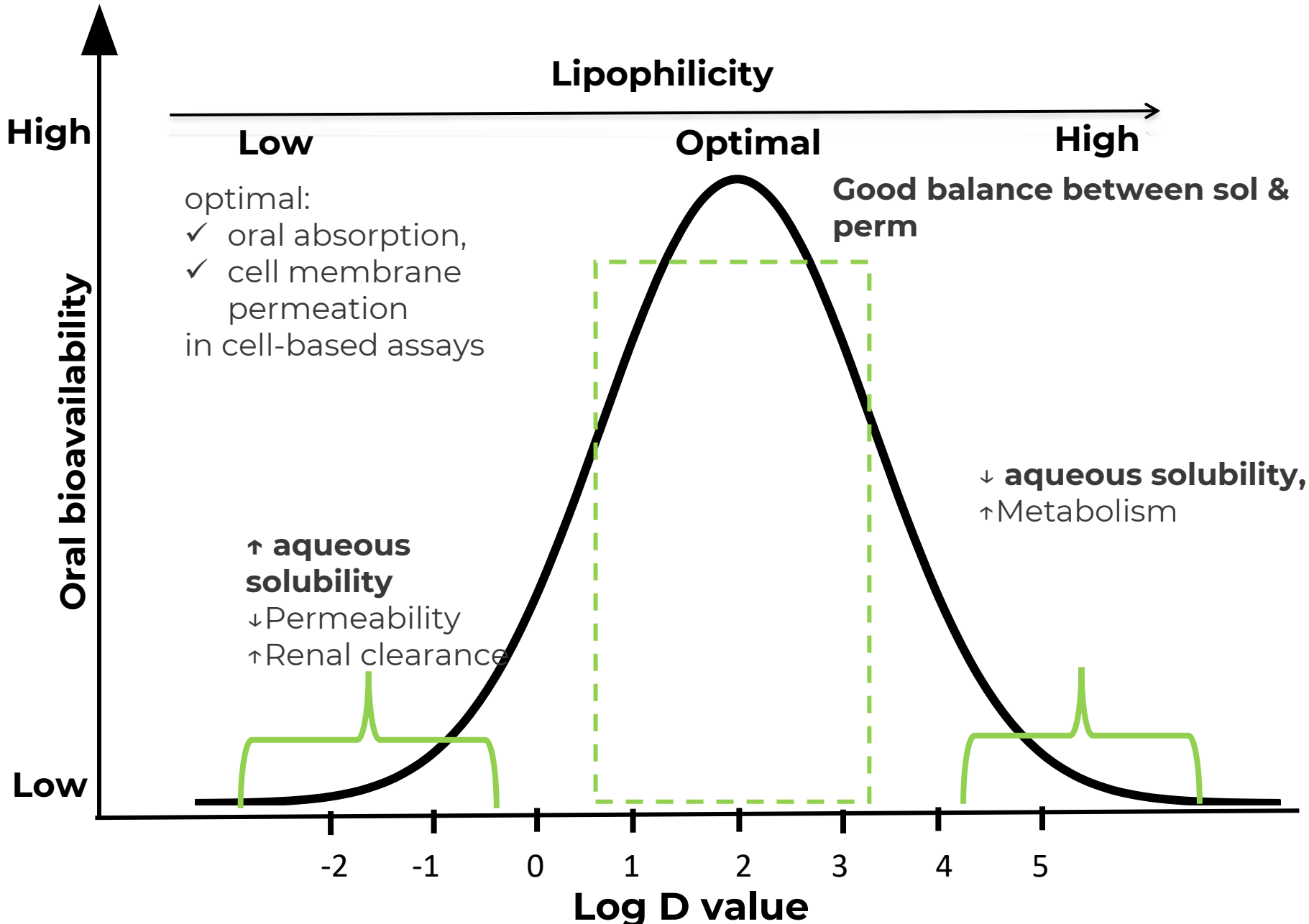
Lipophilicity and pKa

- The distribution of compounds in biological systems is dependent on their ability to partition between polar and non-polar environments.
- This is a function of 2 related properties – lipophilicity and ionization
- Lipophilcity is measured as a partition coefficient (Log P) between an organic phase and an aqueous phase, assuming all molecules are in neutral form
- Most drugs are weak based or weak acids and will therefore be at least partly ionised at physiological pH, therefore Log D is often more informative



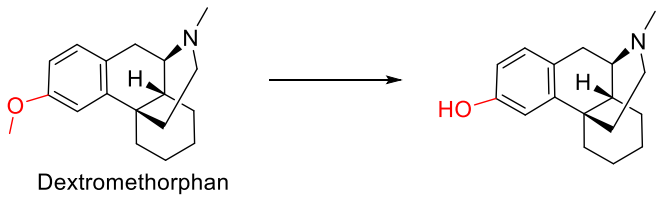
$$\text{Log D} = \text{Log P} - \log\left(1 + 10^{\text{pH} - \text{pKa}}\right) \text{ acids}$$
$$\text{Log D} = \text{Log P} - \log\left(1 + 10^{\text{pKa} - \text{pH}}\right) \text{ bases}$$

Lipophilicity and drug discovery

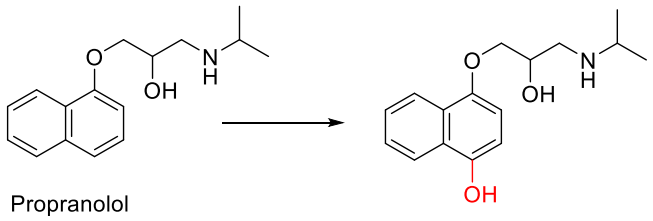


Drug metabolism

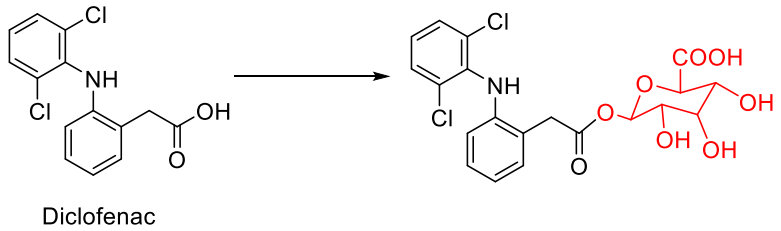
- Enzymatic compound biotransformation, often to more polar, and less active metabolites
- Classically viewed as;
  - Phase I metabolism: Redox reactions to create functional groups (e.g hydroxylation) or expose functional groups already present (Demethylation)
  - Phase II metabolism: Conjugative reactions in which endogenous compounds are attached to functional groups created in Phase I or already present in the molecule



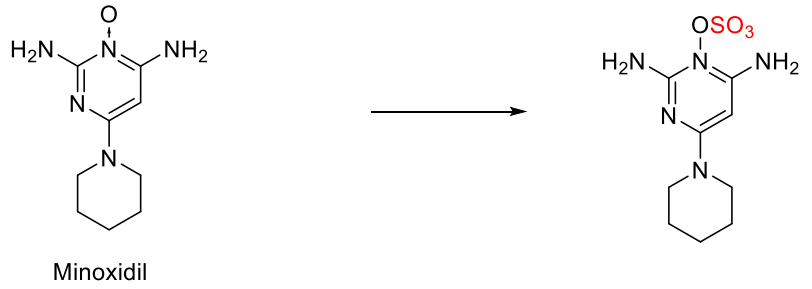
O-dealkylation



Hydroxylation



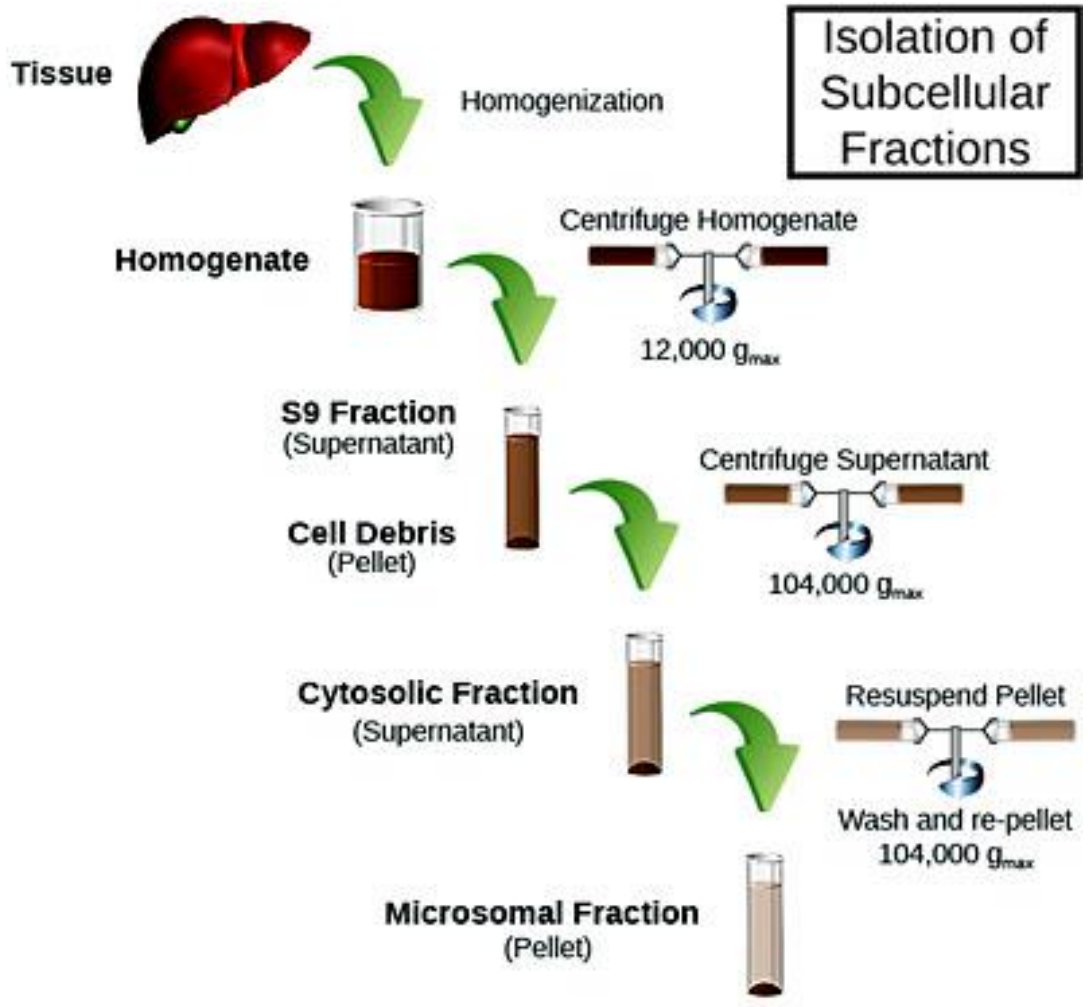
Glucuronidation



Sulfate conjuagtion

Metabolism assays: Metabolic stability

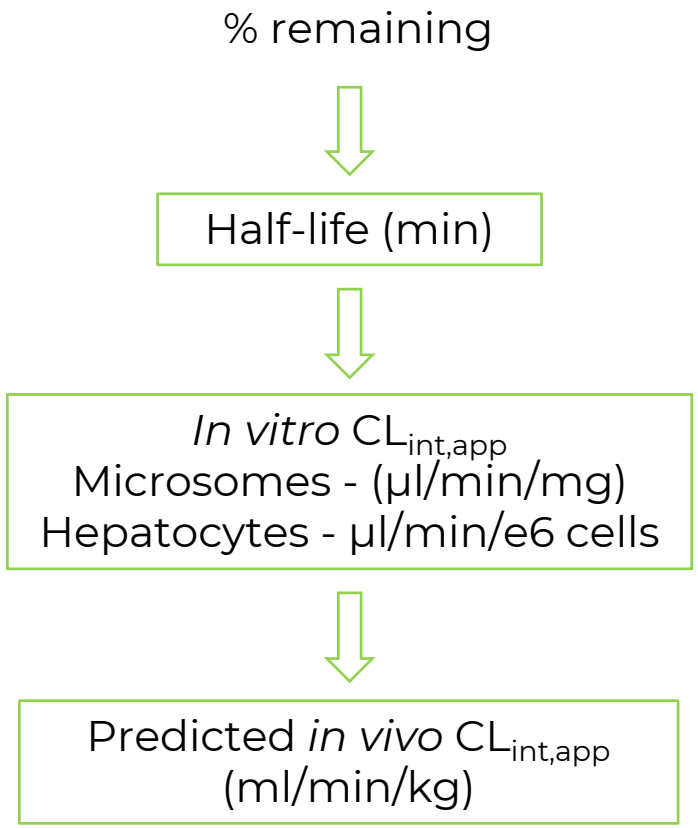
- *In vitro* metabolic stability assays therefore rely on cells and subcellular fractions extracted from the liver:
  - ✓ Hepatocytes
    - Cells prepared by removing connective tissue from the liver
    - More expensive and lower throughput than microsomes
    - Have the full set of enzymes expressed in the liver with both redox enzymes (Phase I metabolism) and conjugative enzymes (Phase II metabolism)
  - ✓ Microsomes
    - Subcellular fractions prepared by homogenisation and ultracentrifugation of liver tissue
    - Offer a cheap high throughput method for screening metabolic stability of compounds
    - Have most of the key enzymes involved in oxidative/reductive metabolism of drugs especially CYP450 enzymes



Sekisui Xenotech©

Metabolic stability data

- *In vitro* metabolism data can be scaled to *in vivo* metabolism by factoring in various scaling factors



Primary data derived from LC-MS analyte responses
Stable – high % remaining e.g >85% in H3D assay

Calculated from % remaining using first order equations
Stable – Long $t_{½}$ e.g >150 min in H3D assay

Apparent intrinsic clearance, calculated $t_{½}$ and incubation parameters
Stable – Low $CL_{int,app}$ e.g <11.6 µl/min/mg or <2.7 µl/min/e6 cells in H3D assay

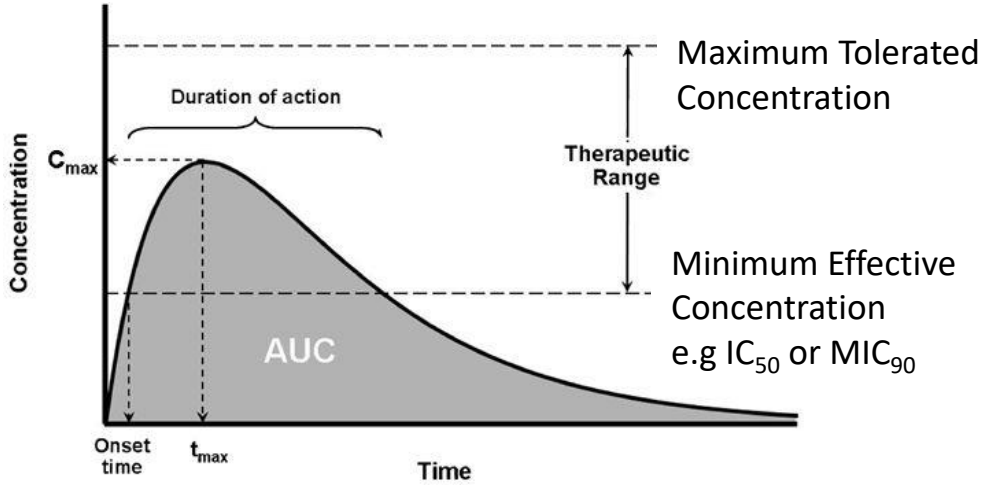
Predicted *in vivo* intrinsic clearance calculated by scaling microsomes/hepatocytes to liver weight per kg body weight
Can also factor in binding and liver blood flow for predicted *in vivo* clearance

From ADME to PK

- The unbound Area Under the Curve (AUC_u) is one of the key measures of how much compound is in circulation

- For an oral drug;

$$AUC_u = F_{abs} \cdot F_{gut} \cdot \frac{Dose}{CL_{int,u}}$$



Fraction absorbed
- Physchem – MW, TPSA, pKA etc
- Solubility
- Lipophilicity
- Permeability

Fraction escaping gut metabolism
- **Metabolic stability**

Unbound intrinsic clearance
- **Metabolic stability**

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Session 3: Pharmacokinetics

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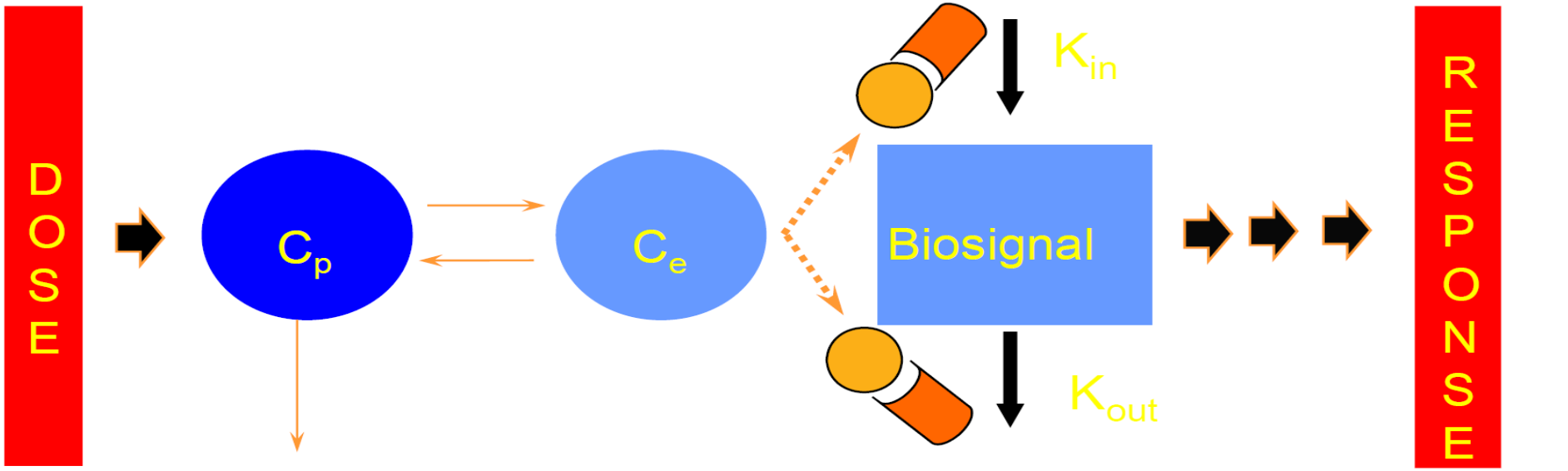
Why do we study PK and the importance of getting the right dose?

Image from http://www.swisstox.ch/

“All things are poison, and nothing is without poison;
only the dose permits something not to be poisonous.”
Paracelsus (1493-1541)

Why do we study PK?

We administer a drug (dose) because we seek a certain effect (response), but a complex chain of events links the administration dose to the observed response



Jusko, Ko, Ebling 1995

Disposition kinetics | Biophase distribution | Biosensor process | Biosignal flux | Trans-duction

Pharmacokinetics (PK) Pharmacodynamics (PD)

Defining PK and PD ?

"Pharmacokinetics may be simply defined as what the body does to the drug,

as opposed to pharmacodynamics which may be defined as what the drug does to the body"

Leslie Z. Benet

Pharmacokinetics describes what happens to the drug after it enters the body, while pharmacodynamic describes its effect.

Let's start with PK

- PK is based on the description of drug concentrations.

The processes that characterize PK are summarized in the (L)ADME scheme.

1. Liberation
2. Absorption
3. Distribution
4. Metabolism
5. Excretion

Elimination

Disposition

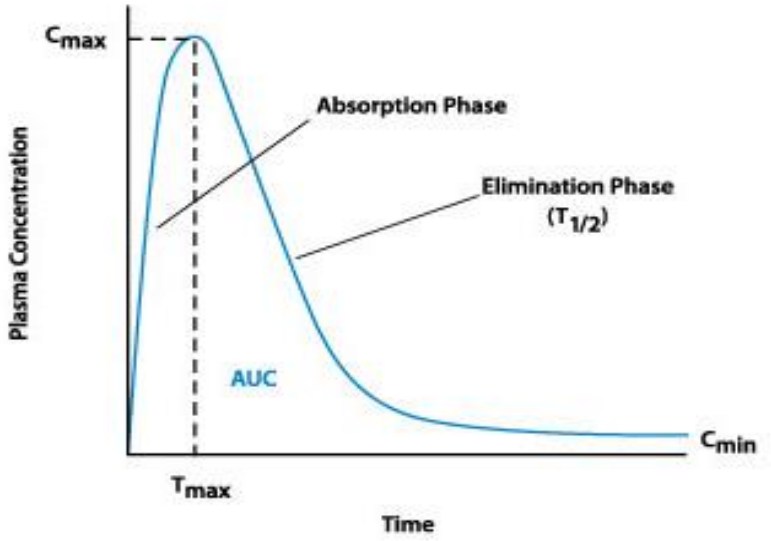


Image from http://saladax.com

Understanding PK concepts

Primary PK parameters	Secondary PK parameters
• Bioavailability • Volume • Clearance	• Elimination half-life • Area Under the Curve (AUC) • Max conc. (C_{max}) • Time of C_{max} (T_{max}) • Steady-state trough conc. (C_{min})
These reflect actual physiological processes and concepts	These are just features of the PK curve, a mere result of the processes underpinned by the primary PK parameters

Absorption

Absorption is the movement of **unchanged** drug from the site of administration into the blood.

Drug absorption is determined by:

Physicochemical properties of the drug

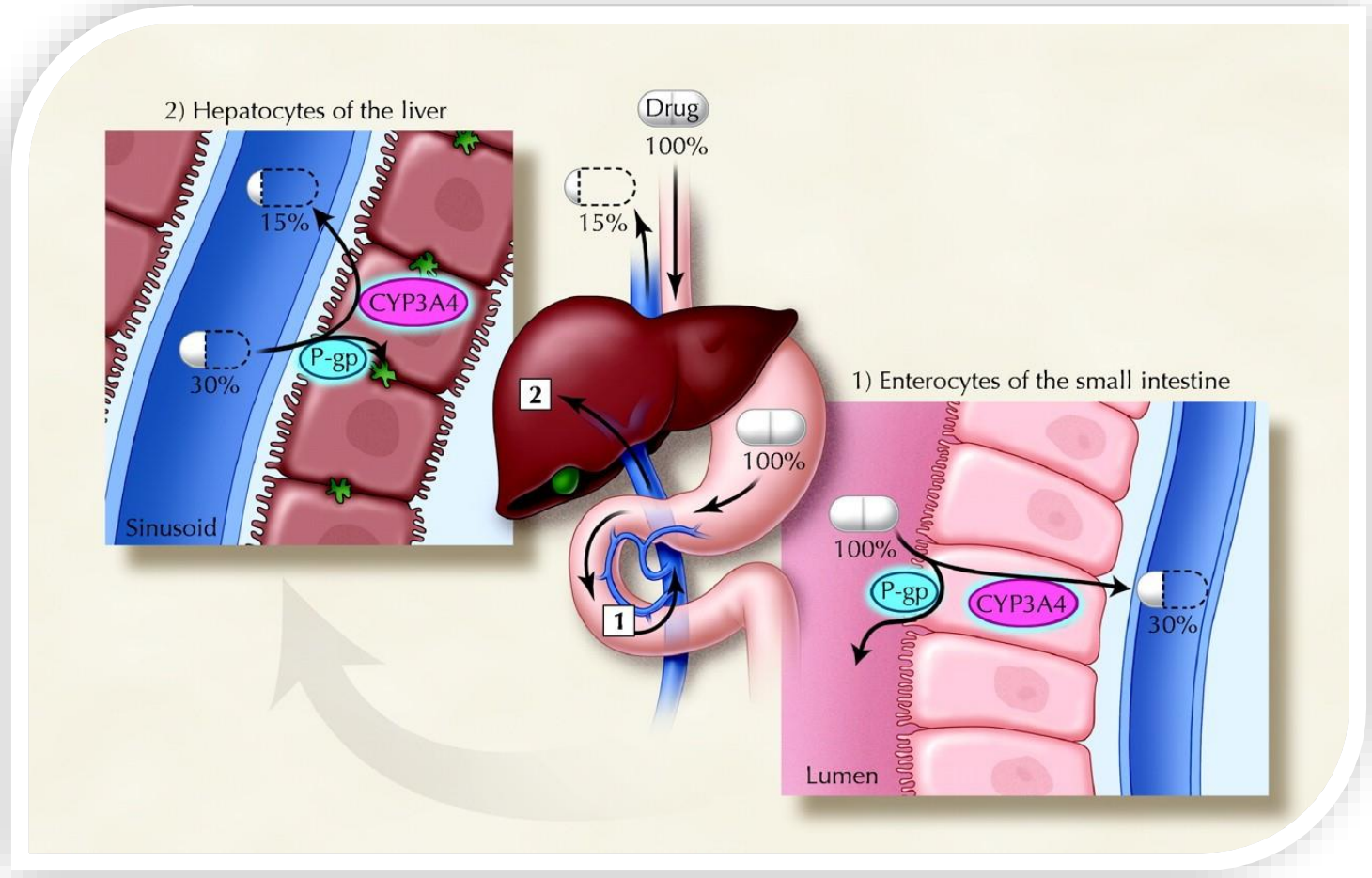
Formulation, for example, tablets, capsules or solutions

Routes of administration

Oral	Sublingual: drug dissolved under the tongue and absorbed through mucous membranes into the bloodstream		Transdermal
Rectal	Inhalation	Intranasal	Parenteral: intravenous, intramuscular and subcutaneous

Drugs must be in solution to be absorbed – Liberation Step, the “L” in (L)ADME

Barriers to Absorption

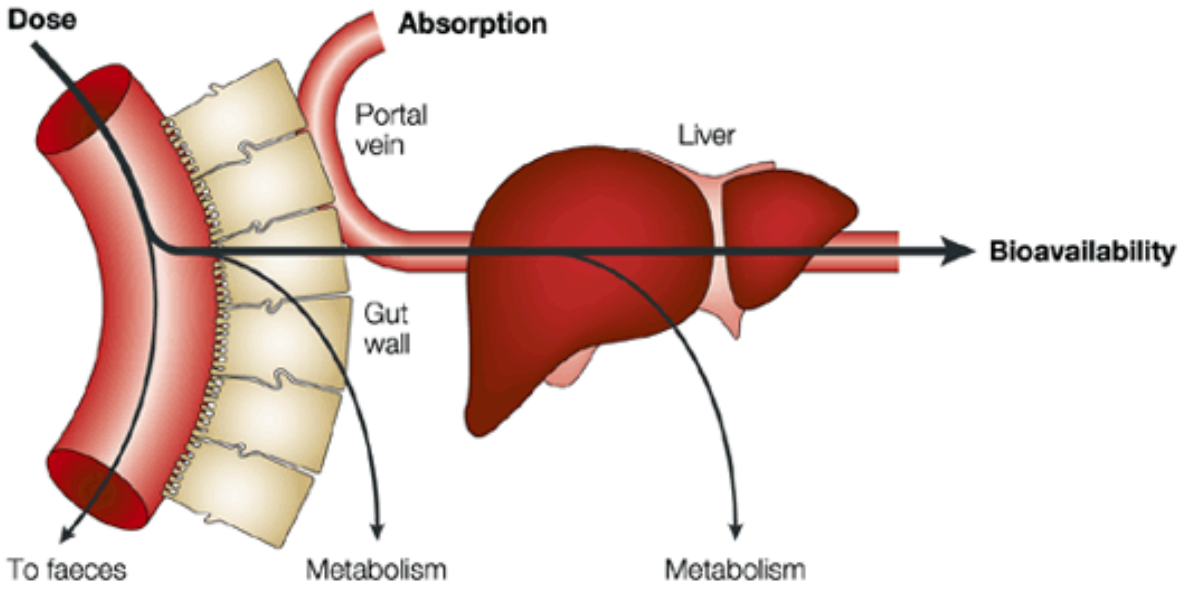


Few potential sites of loss contribute to decrease in systemic absorption

Bioavailability

Bioavailability (F) represent the fraction of intact drug that is systemically available, i.e. reaching the systemic circulation.

For intravenous administration F = 1, for any other route of administration 0 ≤ F ≤ 1

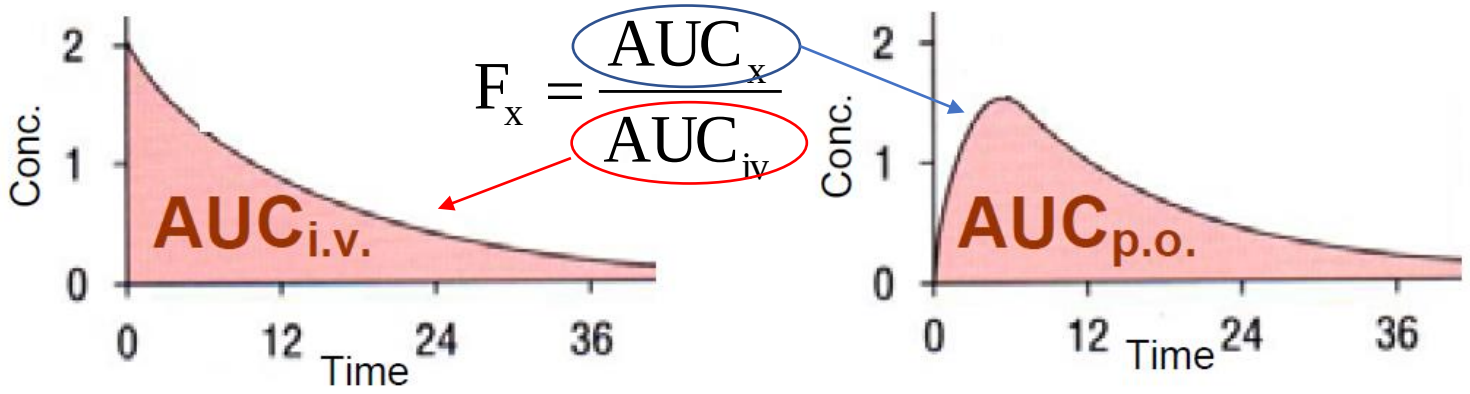


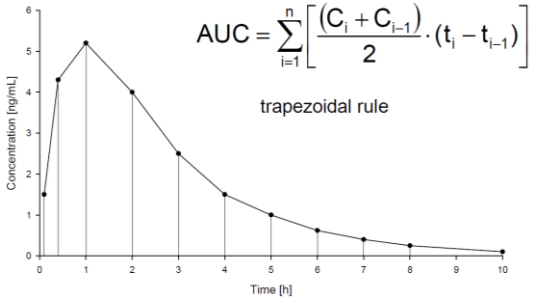
Not all the drug given with an oral dose reaches the site of measurement because of:

- incomplete dissolution
- Incomplete absorption
- pre-systemic metabolism

Bioavailability

To estimate the absolute bioavailability of a certain formulation or route of administration x, it is necessary to compare its exposure (measured as Area Under the concentration-time Curve - AUC), with the exposure of IV administration.





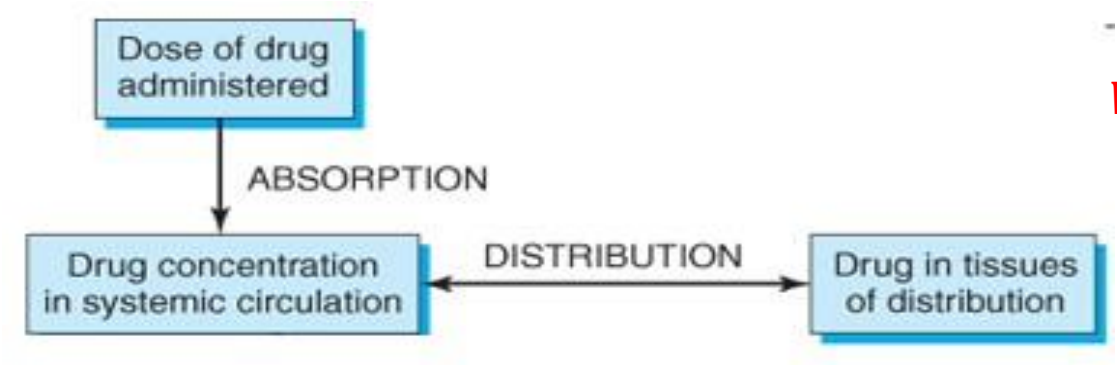
NCA Analysis

BIOAVAILABILITY is clinically relevant for the determination of dose for all non-IV administrations

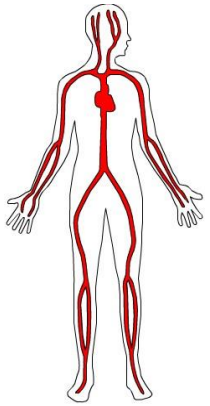
Distribution (D)

The apparent volume of distribution (Vd) quantifies the distribution of the drug in the body.

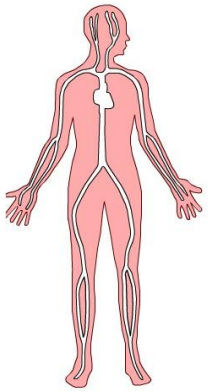
It is a proportionality factor that relates the drug amount in the body and its concentration measured in a biological fluid



$$Vd = \frac{Total\ Amount\ of\ drug\ in\ the\ body}{Measured\ Concentration}$$



It is apparent because it assumes that the drug is evenly distributed throughout the body with the same concentration measured (e.g. blood/plasma).



In humans:
<4 : confined to plasma
4 – 14 L : Throughout the blood (plasma and blood cells)
> 42 L : Tissues most likely fatty tissues

Volume of distribution (Vd)

- Volume of distribution provides little information about the specific pattern of distribution.

- Each drug is uniquely distributed in the body:

Fat	Extracellular fluid	Specific tissues

Volume of distribution

High	Low
• High lipid solubility (non-polar) • Low rates of ionisation • Low plasma binding capabilities	• Polar • More highly ionised • High plasma binding

It is clinically relevant for drug dosage regimen to determine the loading dose

Why is unbound concentration important?

Only <u>unbound</u> drug diffuses into tissues, have pharmacological/toxicological effect and can be eliminated

ALBUMIN - ACIDIC DRUGS

α1- ACID GLYCOPROTEIN - BASIC DRUGS

GLOBULINS - STEROIDS

fu depends on:

Affinity for proteins (Ka)

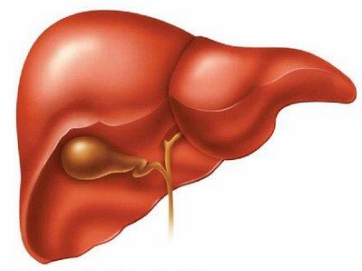
Protein concentration

Drug concentration

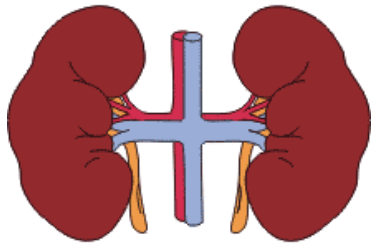
Clearance (CL)

Elimination - ME in the (L)ADME scheme – is normally quantified by clearance

Clearance can be interpreted as the volume of fluid “cleared” of the drug per time unit



- Metabolism
  - main mechanism of drug elimination
  - liver and intestine as major sites
  - metabolites more polar than the parent drug and renally excreted



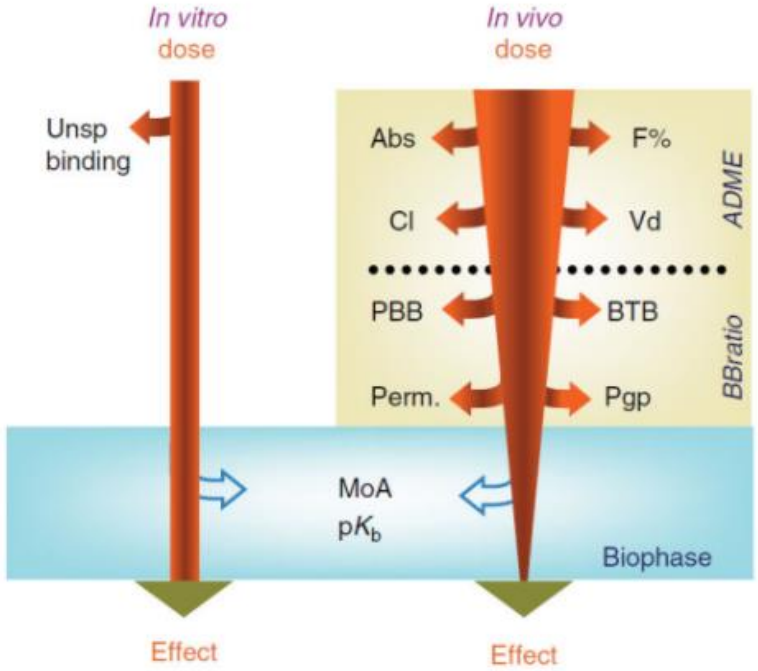
- Excretion
  - kidneys – renal
  - liver – hepatic
  - lungs - pulmonary (volatiles)

$$CL = \frac{Dose}{AUC}$$

mL/min/kg

Mouse: 90 mL/min/kg
Rat: 85 mL/min/kg
Human: 21 mL/min/kg

In vitro activity ≠ In vivo efficacy



Abs: Absorption; **BTB**: Brain Tissue Binding; **Cl**: Clearance; **F**: Bioavailability; **MoA**: Mode of Action; **Perm**: Permeability across membranes; **PBB**: Plasma/Blood protein Binding; **Pgp**: P-glycoprotein interaction; **Vd**: Volume of Distribution; **pKb**: Target affinity