Introduction to and Application of Pharmacokinetics in Pharma R and D

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4th February 2015

Objectives

- Provide an overview of pharmacokinetics (PK), its key processes, parameters, elimination pathways and iv and po PK profiles
- Summarise methods used in PK studies
- Highlight the importance and value of pharmacokinetics
- Define pharmacodynamics (PD) and relationships between PK and PD
- Applications of PK and PD in R and D
  - Dosage regimen design
  - Support to preclinical studies
  - Relevance of drug metabolism
- Drug interactions
- Prediction of clinical PK from preclinical data
- Factors affecting drug disposition
- Clinical pharmacokinetics in drug development
- PK support to Go No Go decision-making

Pharmacokinetics - definitions

- Pharmacokinetics (PK): The time course and movement and fate of drugs in the body i.e. what the body does to the drug
  - Absorption - the process(es) by which drug moves from its site of administration to the site of measurement (usually plasma or blood)
  - Distribution - the reversible transfer of drug from the site of measurement (usually plasma or blood)
  - Elimination - the irreversible transfer of drug from the site of measurement (usually plasma or blood) and includes:
    - Metabolism - chemical alteration of a drug by a biological system with the principle purpose of eliminating it
    - Excretion – removal of the unchanged drug or a metabolite in urine, bile, lungs (for volatile drug or metabolite), sweat, breast milk
  - PK parameters
    - Clearance (Cl_{tot}) - volume of plasma, blood or serum completely cleared of total or unbound drug/unit time (ml/min or L/H)

      $$Cl_{tot} = Cl_{renal} + C_{metab} + Cl_{biliary} + \ldots.$$

    - Volume of distribution (V_D) - relates drug concentration in plasma, blood or serum to the amount of drug in the body
    - Half-life ($t_{1/2}$) - time taken for drug concentration in plasma, blood or serum by one half

      $$t_{1/2} = \frac{0.693 \times V}{Cl_{tot}}$$

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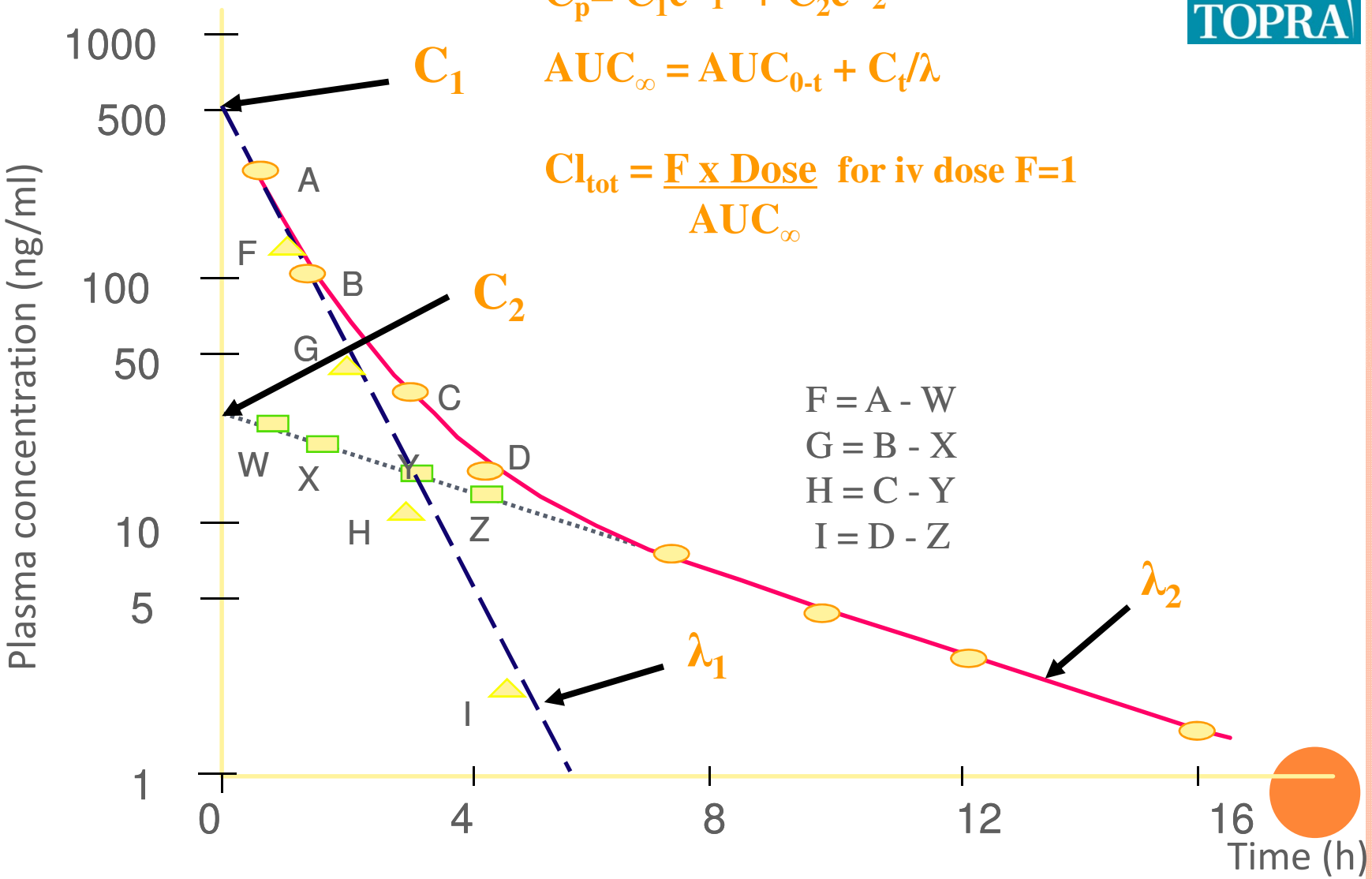
Elimination Pathways

- Excretion of drug and/or metabolites in urine, bile and/or faeces
- Metabolism of the drug and/or primary metabolite(s)
  - Phase I reactions produce or introduce a new chemical group in a drug molecule
    - Oxidation
    - Reduction
    - Hydrolysis
  - Phase II or conjugation reactions involve the linking of the drug to an endogenous molecule
    - Glucuronidation
    - Sulphation
    - Amino acid/glutathione
    - N-acetylation

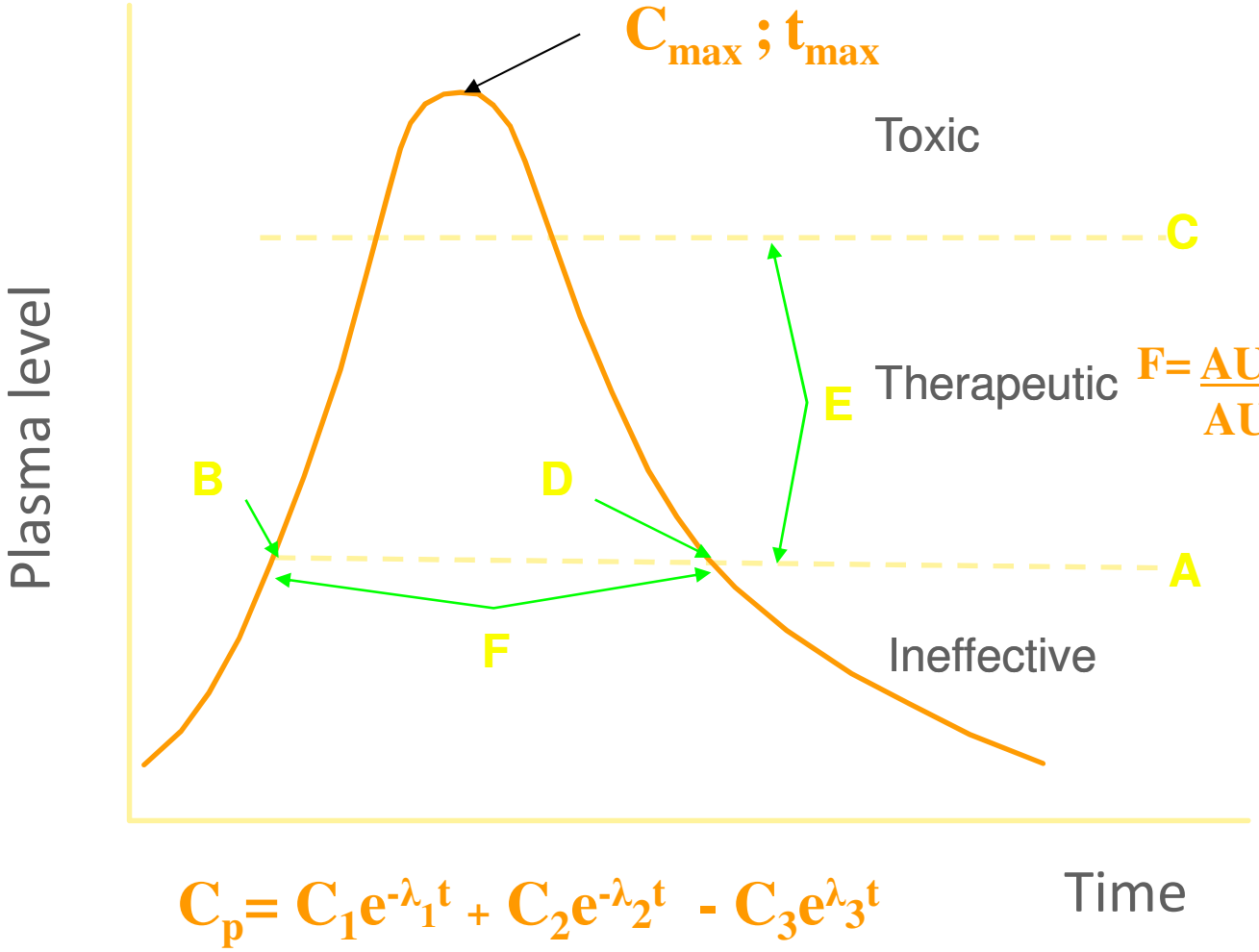
Pharmacokinetics and Drug Metabolism Techniques

- ***In vitro***
  - Microsomes: rat, dog, monkey, human
  - Isolated expressed cytochromes, tissue homogenates,
  - Primary hepatocytes
  - Cell cultures, tissue slices, isolated perfused organ(s)
- ***In vivo***
  - Whole animal: rat, dog, monkey, human
- ***Bioanalytical techniques***
  - LC-MS, LC-MS/MS, HPLC-UV, immunoassay
  - LC-NMR, LC-MS
  - Radioisotope Techniques

PK: Multi-exponential i.v. plasma concentration-time curve



PK: Time Course Of Orally Administered Drug



$$F = \frac{AUC_{po} \times Dose_{iv}}{AUC_{iv} \times Dose_{po}}$$

$$C_p = C_1 e^{-\lambda_1 t} + C_2 e^{-\lambda_2 t} - C_3 e^{\lambda_3 t}$$

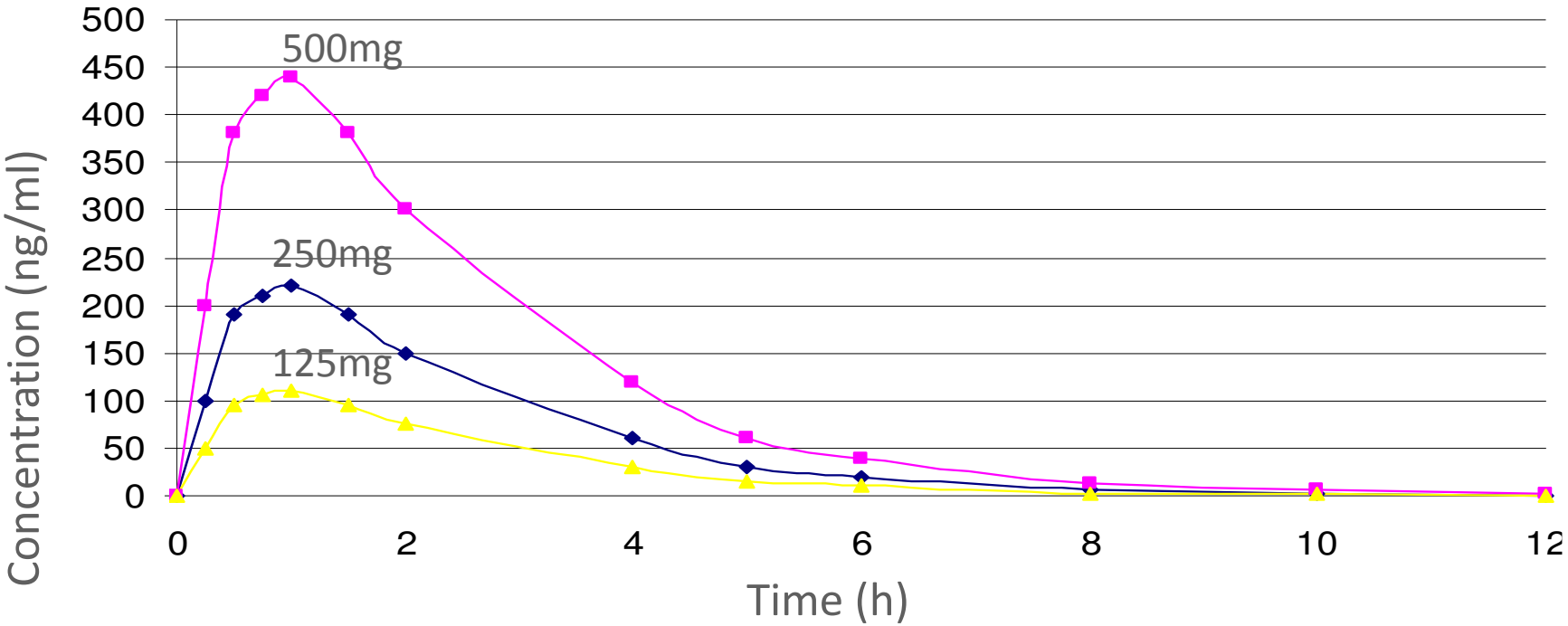
Critical PK/ADME Questions 1

- How much of the drug is absorbed, how much reaches the systemic circulation and how quickly (absorption/bioavailability)?
- Does the drug reach the site of action and what organs are exposed to the drug and/or metabolites (distribution)?
- How long does it stay in the body, how is it removed and are there any active metabolites (elimination)?
- Are the pharmacokinetics of the drug linear with dose and time?
- What factors affect drug disposition?
- What are the most appropriate route(s) and means of administration?
- Are these characteristics consistent with the clinical and commercial objectives?

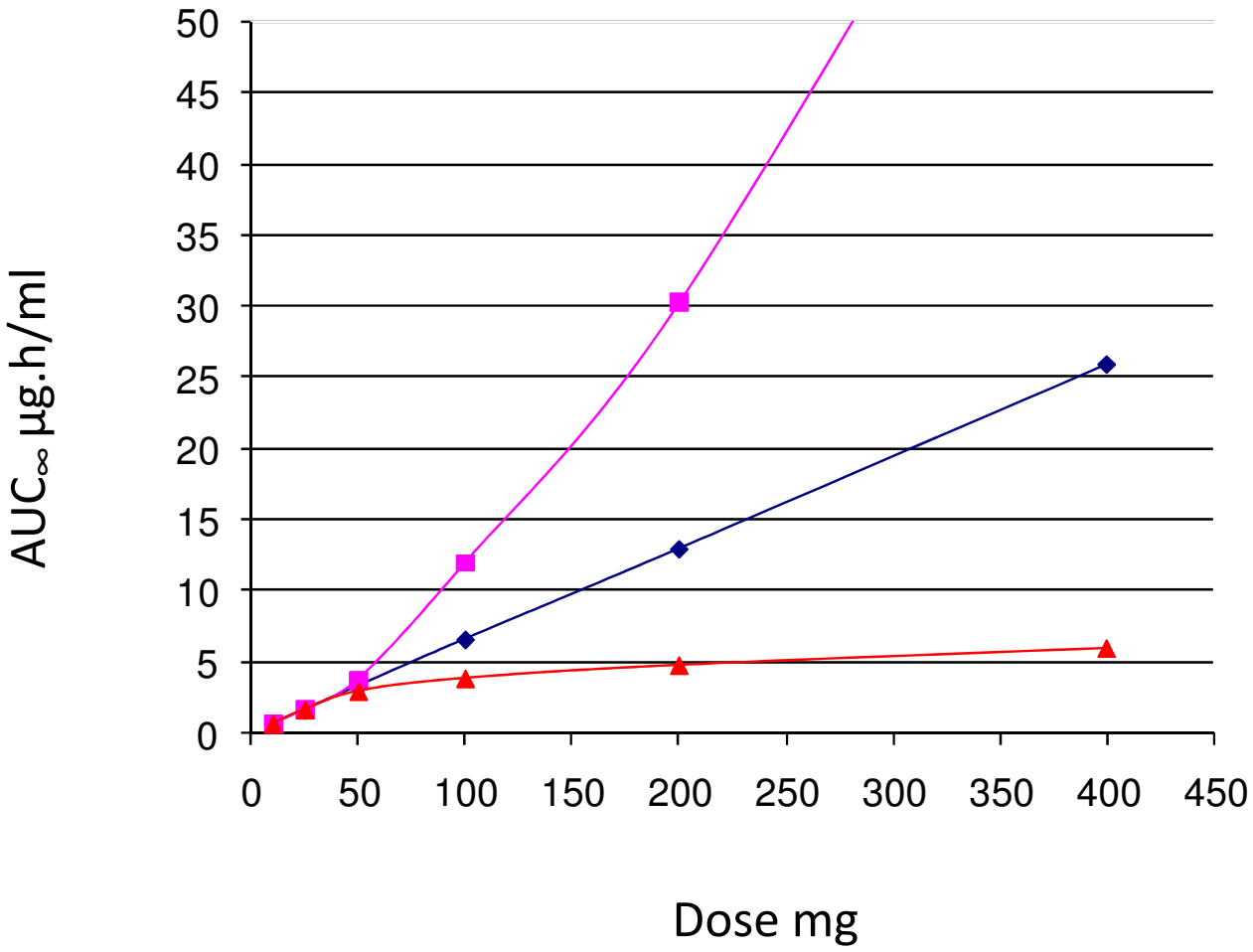
Critical PK/ADME Questions 2

- What are the appropriate doses/dosage regimens for:
  - Animal pharmacology/toxicology?
  - Healthy volunteers?
  - Patients….general, sub-groups, individuals?
- Which drug interactions are likely to be important determinants of clinical acceptability and use?
  - Caused by the new drug?
  - Exerted on the new drug?
- Why have you adopted the development strategy?
- What are the key features determining the understanding of how this drug is handled by the body?
- Do you fully understand the relationship between the formulation/absorption/disposition and the desired/adverse affects?

Single oral dose PK profiles in man



Dose Linearity of Pharmacokinetics



$$Cl_{tot} = \frac{F \times Dose}{AUC_{\infty}}$$

Influence of Physico-chemical properties on Drug Disposition

Property	Influence
Structure	Partition/distribution coefficient, pK_a acid/base/neutral stability,
Partition Coefficient (logP) Distribution Coefficient (logD)	Solubility, absorption, binding, distribution, elimination
pK_a, acid/base/neutral	Absorption, distribution, elimination
Molecular weight	Membrane transport Biliary excretion

Pharmacodynamics - definitions

- Pharmacodynamics (PD): The time course and intensity of drug action in the body i.e. what the drug does to the body, includes wanted and unwanted effects
  - Pharmacology
  - Toxicology
  - Adverse events/side effects
- Based on:
  - Dose or concentration/response curves
  - Log dose/response curves
  - Log concentration/response curves
  - E_{max} model
  - Concentration/E_{max} model
- There is a relationship between PK and PD
  - No drug concentration; no effects
  - Optimal drug concentration; optimal PD with low AEs/toxicity i.e. safe and well tolerated PD
  - Excessive drug concentration; maximum PD with marked toxicity

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Pharmacodynamics

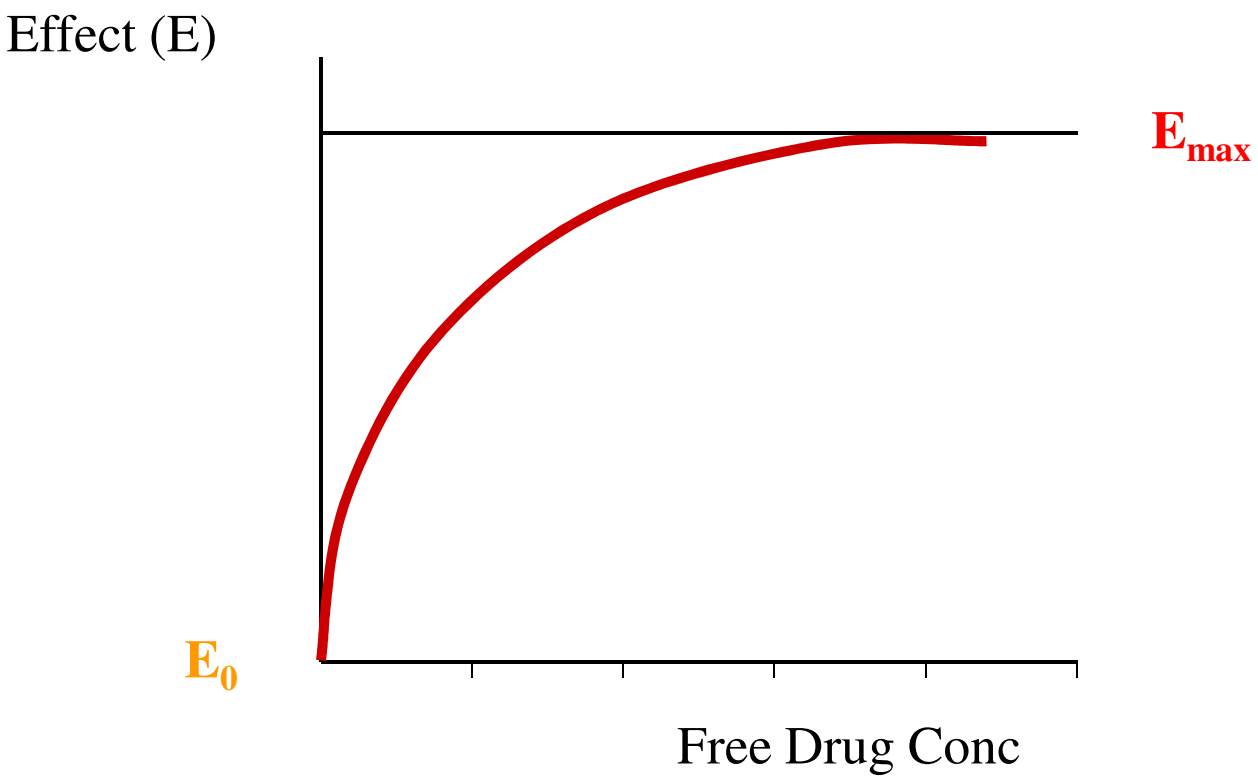
Drug Receptor Interaction

Michaelis-Menten Equation:

$$\text{Effect} = \frac{\text{Maximal Effect}(E_{max}) \cdot C}{K_D + C}$$

Where K_D = dissociation constant or IC_{50}; C= Free drug concentration

Parameters from E max curves – cartesian



Effect (E) e.g. HR, BP, %Enzyme inhibition

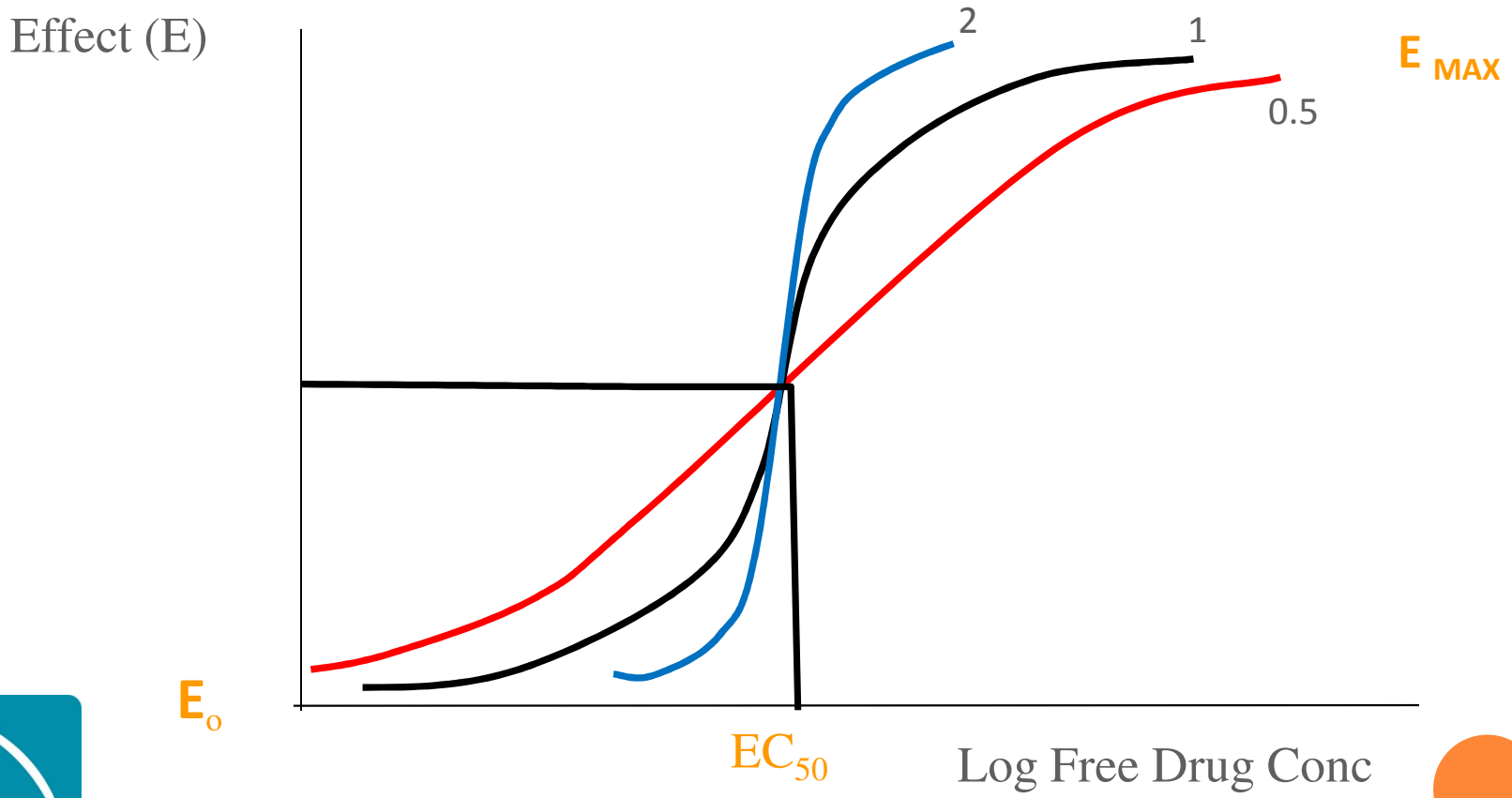
PK/PD Modelling

$$E = \frac{E_{max} \cdot C^N}{EC_{50}{}^N + C^N} + E_0$$

Where:
E is the effect measured; E_0 at zero time; E_{max} maximal effect
C is the concentration producing the effect
N is the slope of the log conc/response curve

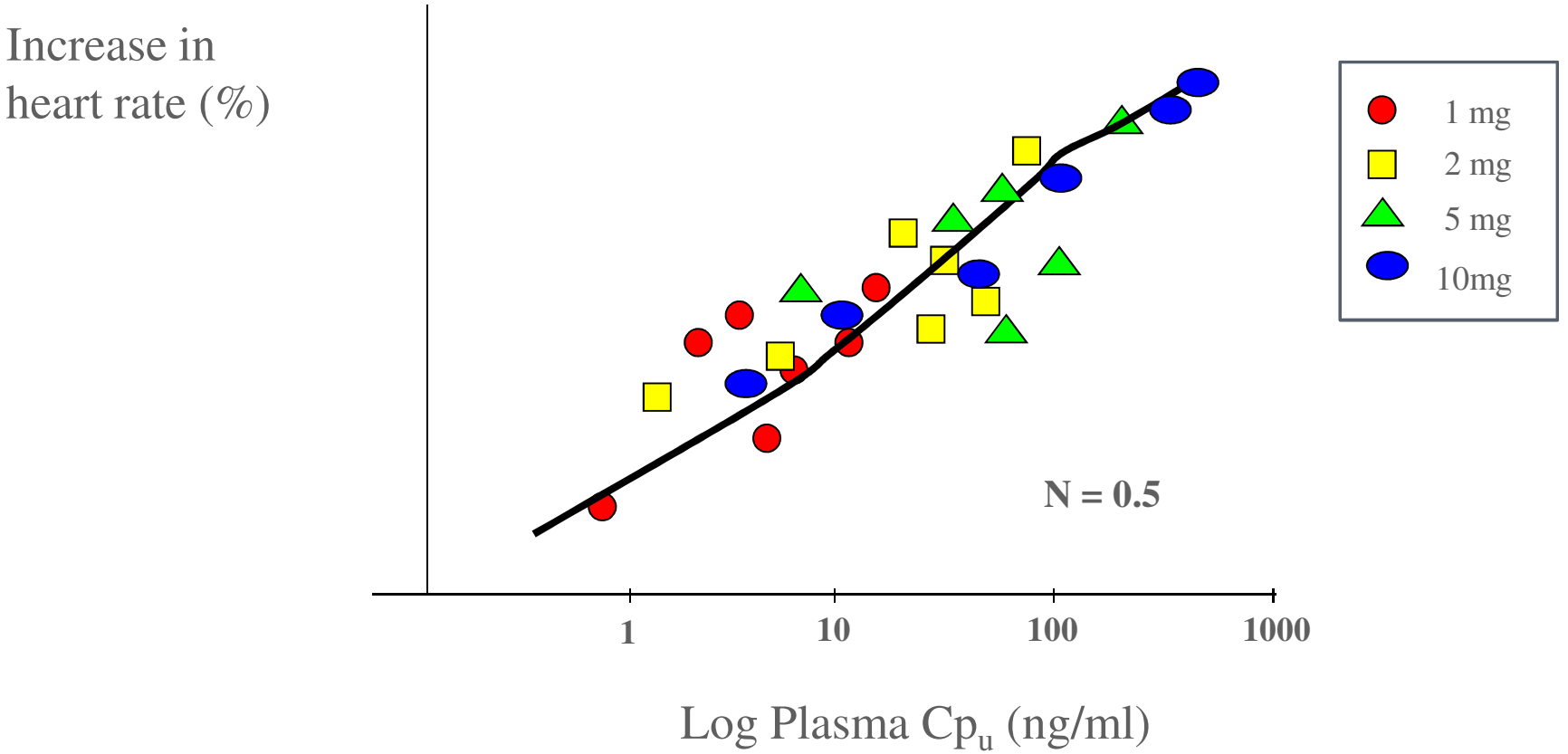
Efficacy will increase faster with concentration at greater N values

Parameters from Emax curves - effect of slope (N)



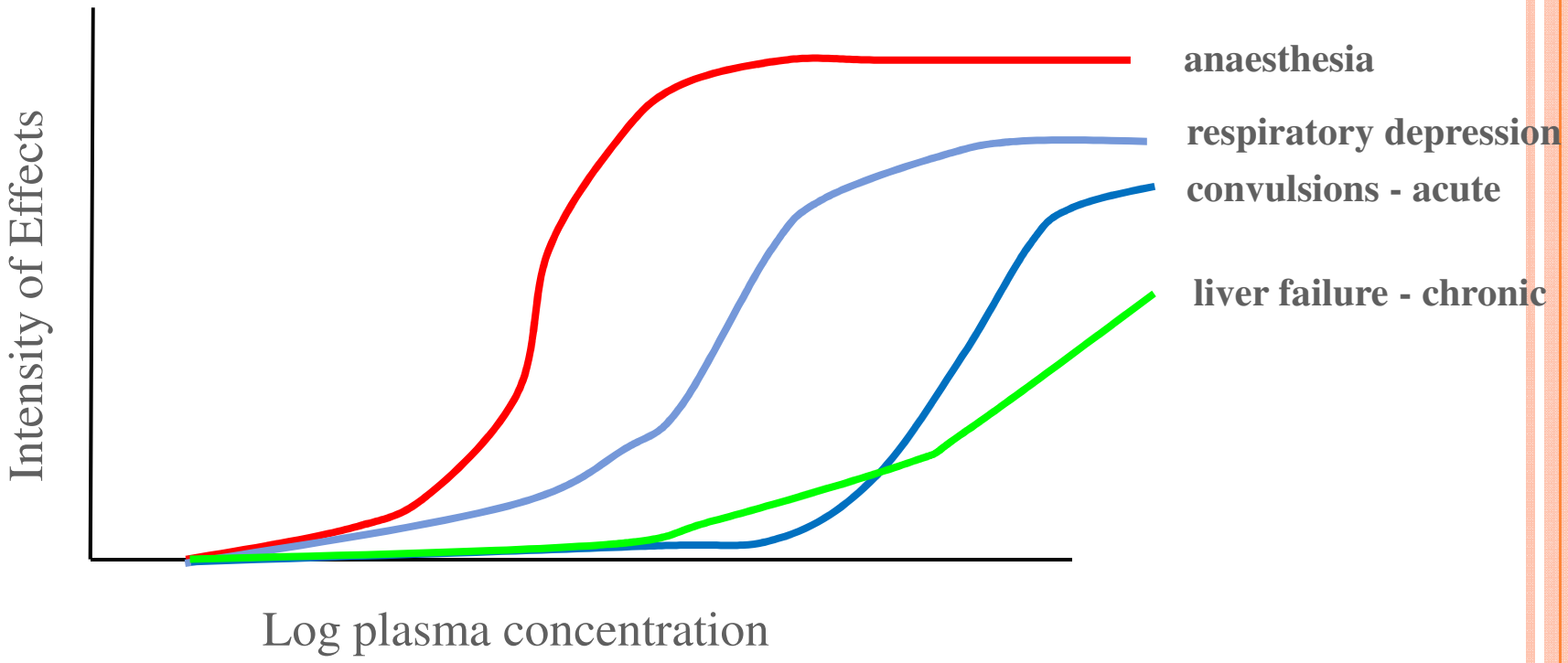
Effect (E) e.g. HR, BP, %Enzyme inhibition

Relationship between Dose/PK/PD for tertatolol



Poor correlation between dose and effect, good correlation between plasma free drug concentration (exposure) and effect

Pharmacology/Toxicology: Log concentration vs response curves

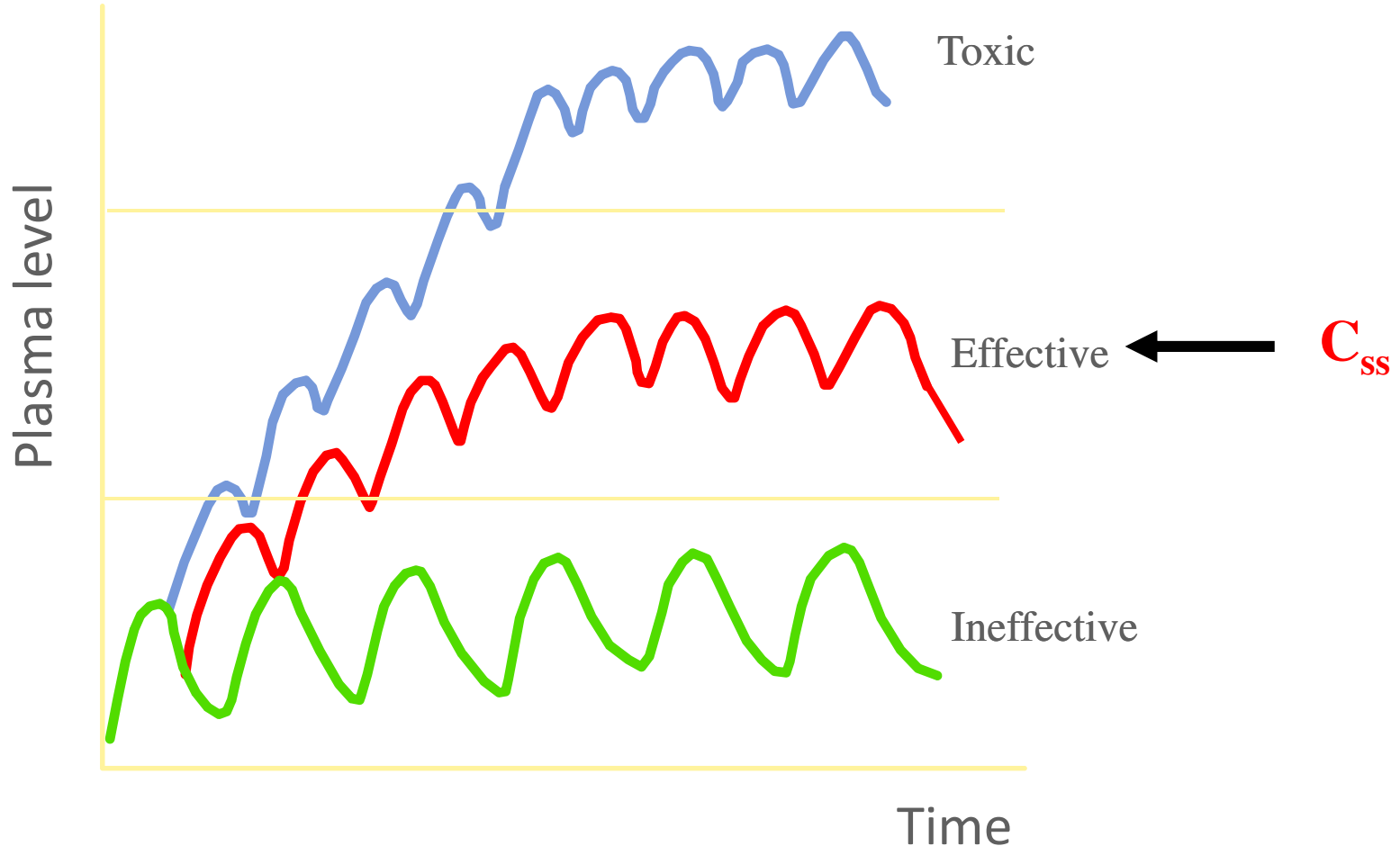


Based preclinical and clinical PK/PD predict dose/exposure/response relationships, predict optimal target concentrations and monitor accordingly

PK and PD Objectives

At steady-state Rate in = Rate out α Cp_{ss} = Cl_{tot} x Cp_{ss} = Cl_{tot}/F x Cp_{ss}

Time to steady-state is ~ 4 $t_{1/2}$



When and what for Preclinical Pharmacokinetics

When	Which	What	Why
Drug Discovery	Rat/mouse/dog/ (monkey)/man	*In vitro* PK/ADME in Caco-2 cells/ microsomes/hepatocytes	Absorption potential, metabolism rates, routes, enzymes
Drug Selection	Rat/dog/(monkey)	*In vivo* plasma PK po/iv	Clearance/$t_{1/2}$/absorption/ bioavailability
Pharmacology	Rat/mouse/guinea pig/ dog/(monkey)	Plasma/tissue concentrations	Establish PK/PD relationships
Toxicology Reprotoxicology	Rat/mouse/dog/rabbit/ (monkey)	Toxicokinetics ^{14}C-ADME Metabolite profiles/identification	PK exposure/PK/PD Tissue distribution Excretion balance Elimination routes Enterohepatic circulation Milk/placental transfer Species validation
Long term carcinogenicity	Rat, dog, mouse	Toxicokinetics	Exposure, AUC

Relevance of Drug Metabolism to Drug Action

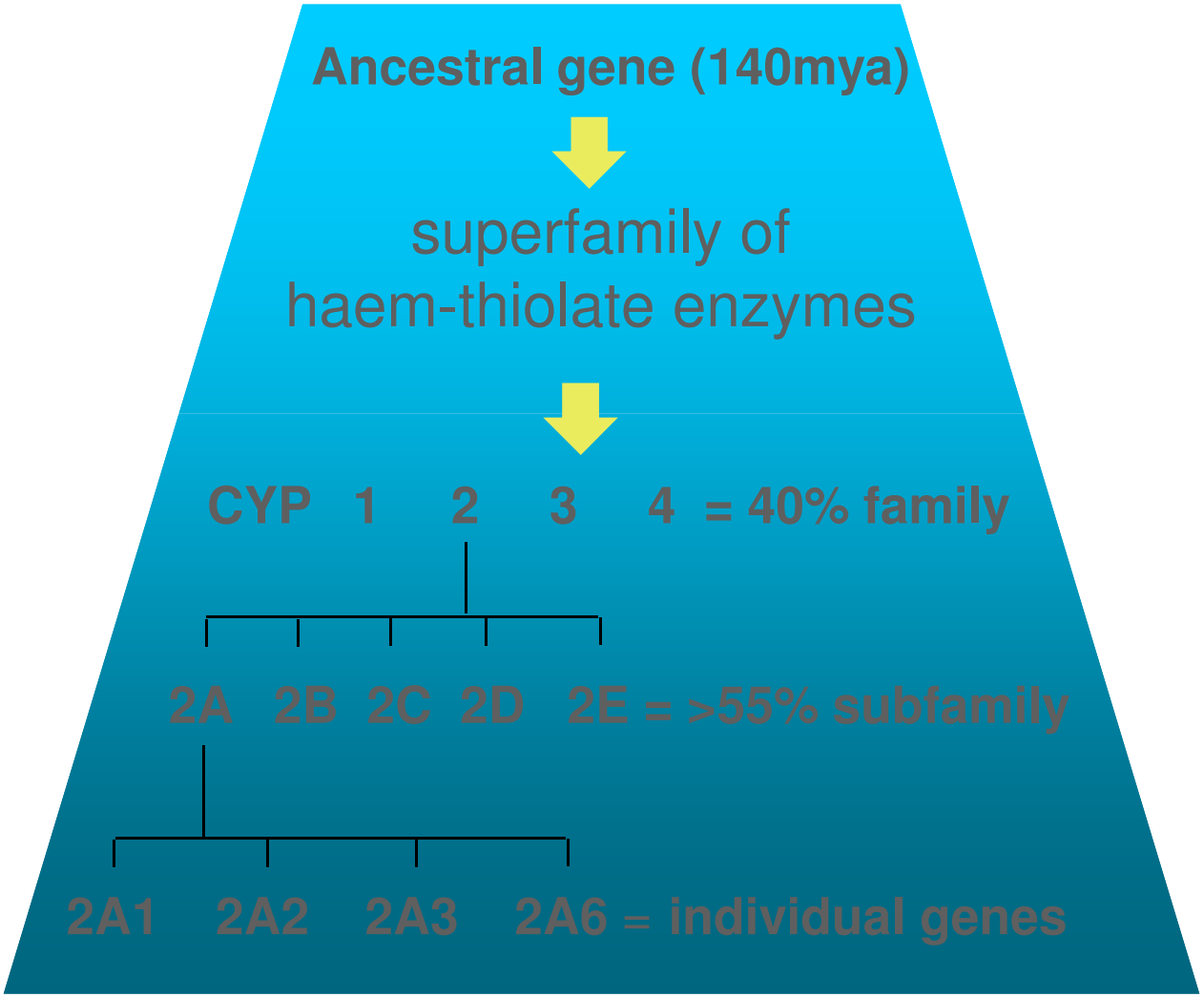
Drug	→	**Metabolite**
Pharmacologically active		Inert
Toxic		Pharmacologically active
Inert (Prodrug)		Toxic

- Relating pharmacodynamics to the fate of the drug
- Interspecies comparison to support interpretation of pharmacology and toxicology
- Drug design
- Lead optimisation

Drug Metabolism

- **What are the metabolic pathways?**
- **Which enzymes systems are involved?**
- **Is a genetic polymorphism involved?**
- **Is the drug an inducer or inhibitor?**
- **What are the possible drug interactions?**

CYP450 Nomenclature



Information for Specific CYP-450 Isozymes

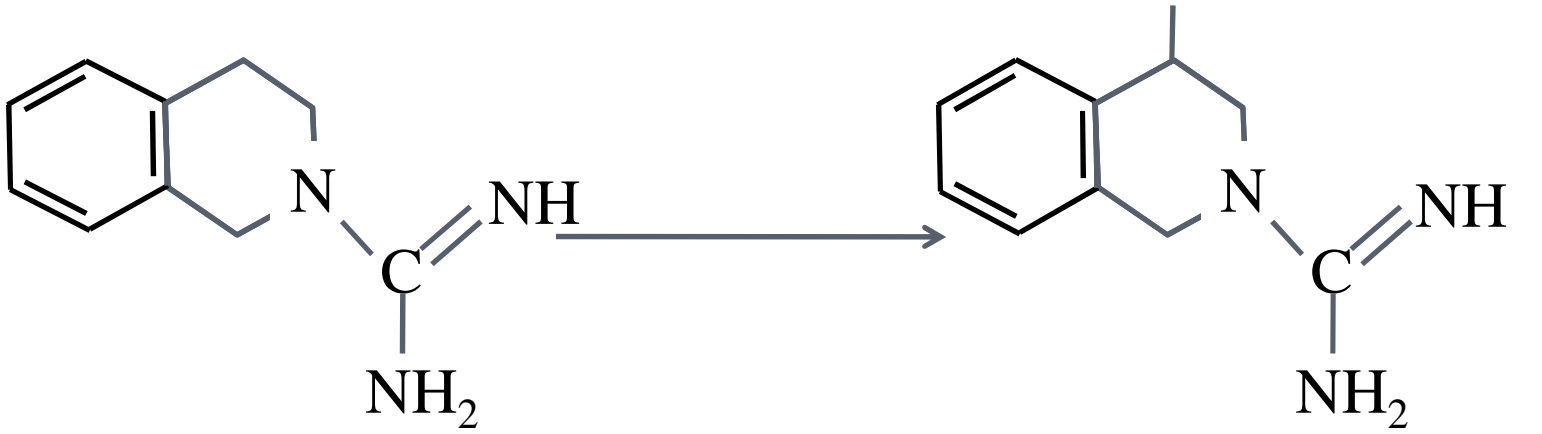
CYP	Compounds	Hepatic Level (%)	Inhibition	Induction	Polymorphism
1A 1/2	Caffeine Biphenols	13	✓	✓ (smoking)	✓(ethnic groups)
2A	Coumarin	4	✓	✓	
2C9/19	Tolbutamide Phenytoin	18	✓✓✓	✓	✓✓ 5%Cauc 23% Oriental
2D6	β-blockers Antidepressants	2	✓✓✓	-	✓✓✓ 5-10% Cauc
2E1	*p*-Nitrophenol Paracetamol	7	✓	✓ (ethanol)	✓
3A4	Many compounds	30	✓✓✓ (grapefruit)	✓✓	Variability (X80)

Which CYP-450 metabolises my drug?

- Incubate drug with microsomes alone and with compounds that specifically inhibit a particular CYP

CYP Isoform	CYP Inhibitor	Km (μM)
1A2	Furafylline	1 – 10
2C	Sulphaphenazole	1 – 5
2D6	Quinidine	1 – 10
2E1	Diethyldithiocarbamate (DDC)	15 – 30
3A4	Ketoconazole Troleandomycin	<2 20 - 200

Genetic Differences



Debrisoquine

4-Hydroxy-debrisoquine

Genetic polymorphism(s)

- 5 - 10% of Caucasians lack the CYP2D6 gene controlling the hydroxylation (poor metaboliser = PMs)
- Individuals on anti-epileptics behave as PMs due to DDI

Side Effects related to CYP-450 2D6 Polymorphisms

Drug Example	Safety Issue/Concern
Debrisoquine	Postural hypotension & physical collapse
Sparteine	Oxytoxic effects
Perphenazine	Extrapyramidal symptoms
Flecainide	Pro-arrythmic effects
Perhexiline	Peripheral neuropathy & hepatotoxicity
Phenformin	Lactic acidosis
Propafenone	CNS toxicity
Metoprolol	Loss of cardioselectivity
Nortriptyline	Hypotension & confusion
Ecstacy	Death

CYP-450 Inhibition/Induction by Drugs

Induction

- Determine the ability of the drug to induce drug metabolising enzymes
- Incubate drug at a range concentrations with human hepatocytes and measure enzyme levels/activity of specific enzymes e.g. 3A4, 2C9/19, 1A2 vs activity of known inducers
- Induction ≥ 40% of known inducers suggests in vivo effects may occur

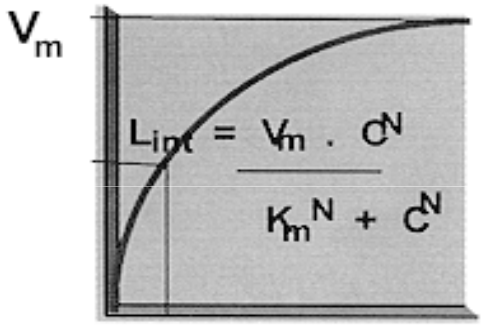
Inhibition

- Determine effect metabolic clearance of other drugs
- Incubate drug with specific substrates of CYPs as shown below
- Determine concentrations likely to cause significant inhibition and compare with target C_{ss} values

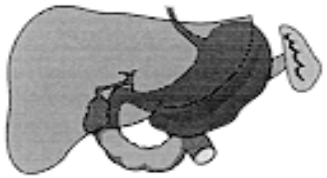
CYP-450 Isoform	Marker Substrate
1A2	Phenacetin O-Deethylation
2A6	Coumarin Hydroxylation
2C9	Tolbutamide Hydroxylation
2C19	S-Mephenytoin Hydroxylation
2D6	Dextromethorphan Demethylation
2E1	Chlorzoxazone Hydroxylation
3A4	Testosterone 6ß-Hydroxylation

Integration of *in vitro* and *in vivo* scaling for prediction to man

	System	Measurements
1. In vitro metabolism	Microsomes Hepatocytes Hepatic slices	Rate calculations

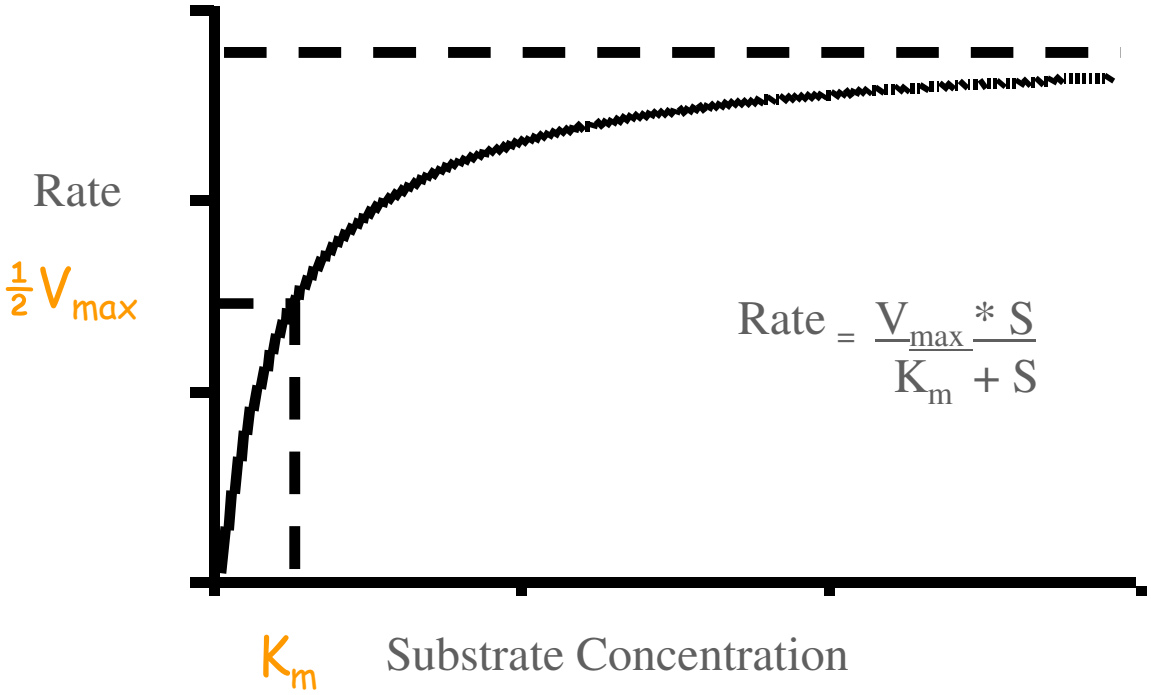


2. Scale up for whole liver



	Liver
Microsomes	500 mg protein/g
Hepatocytes	150 x 10^6 cells /g
Hepatic slices	500 slices (3.5 mg protein)

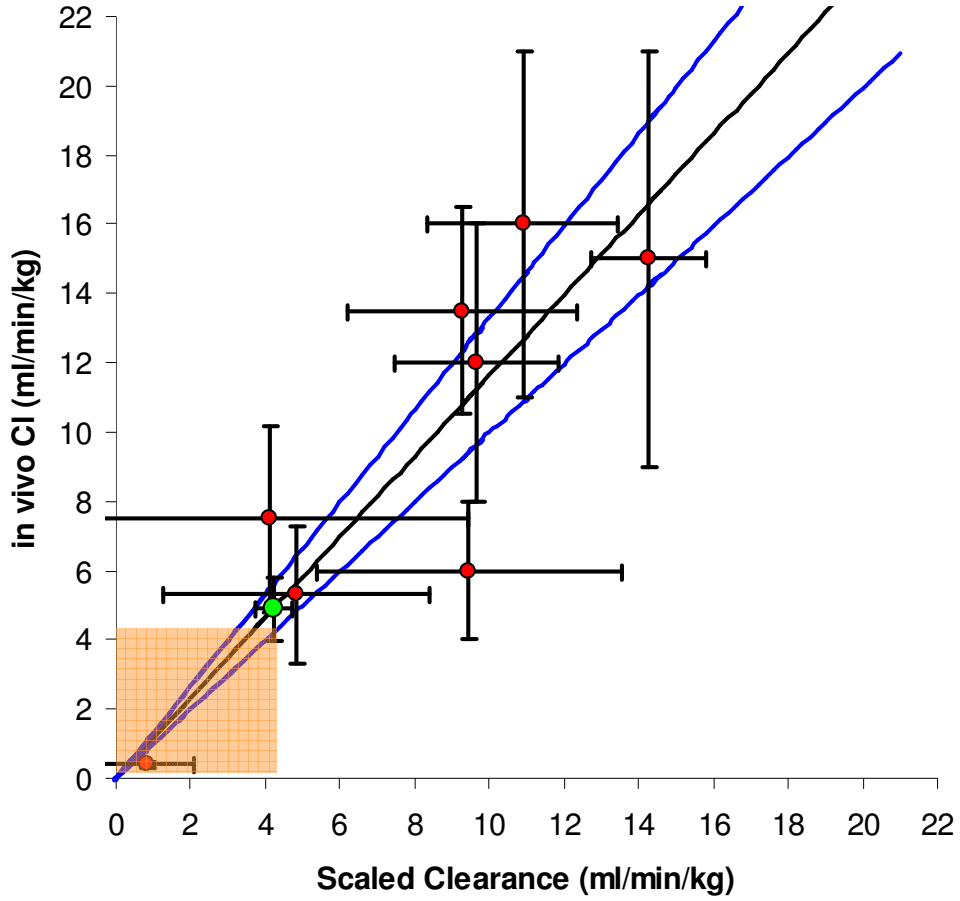
Rate of Metabolism (Cl_{int})



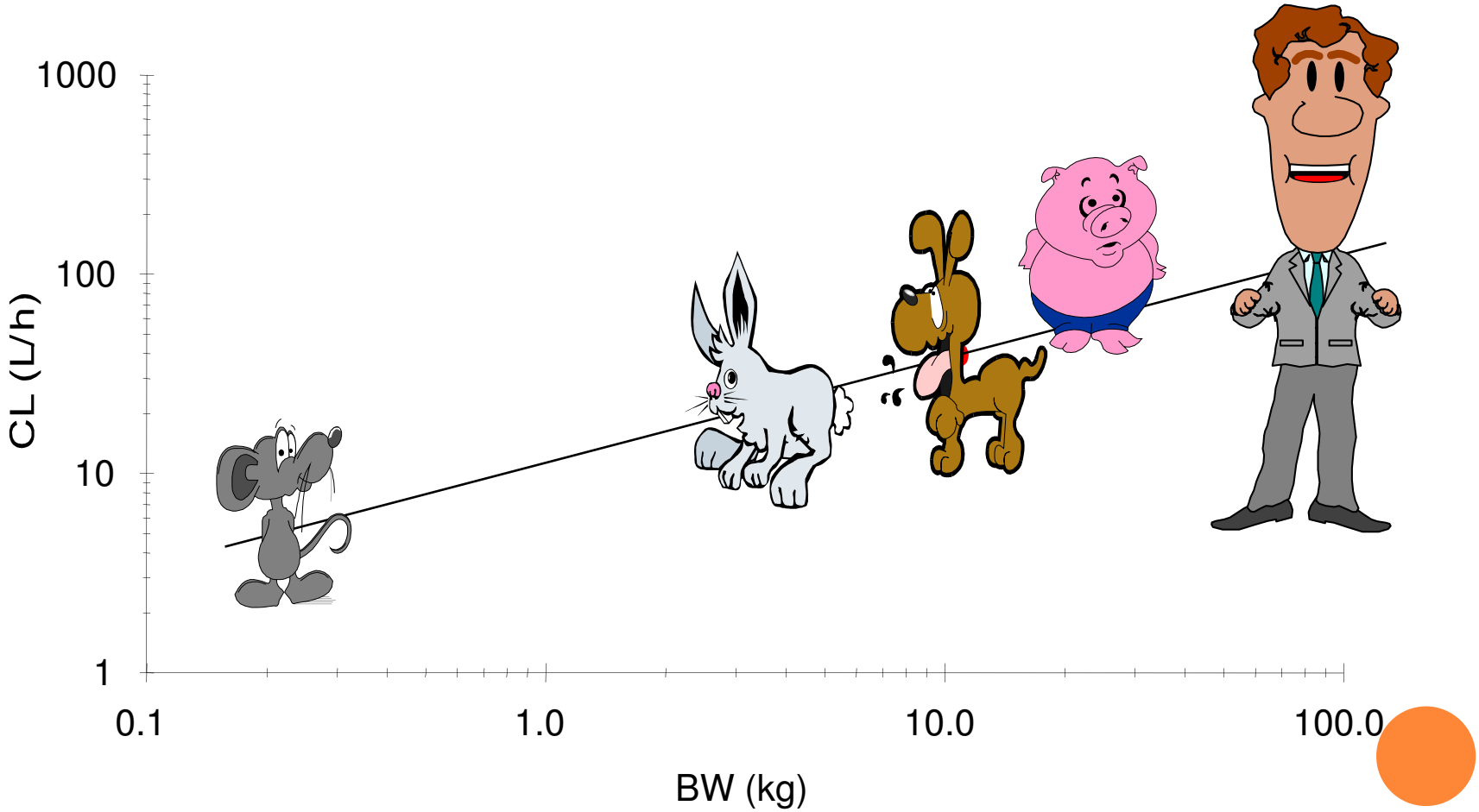
In vitro $Cl_{int} = V_{max}/K_m$ at low [µM]

$$\text{In Vivo Cl} = \frac{\text{Fu.Clint.Qh}}{\text{Fu.Clint + Qh}}$$

Predicting human clearance by *in vitro-in vivo* scaling

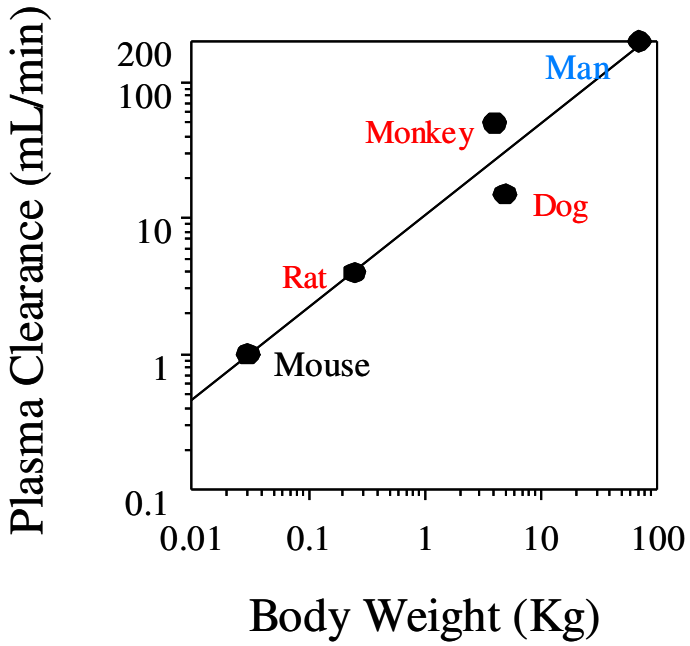


Dose scaling - Allometry



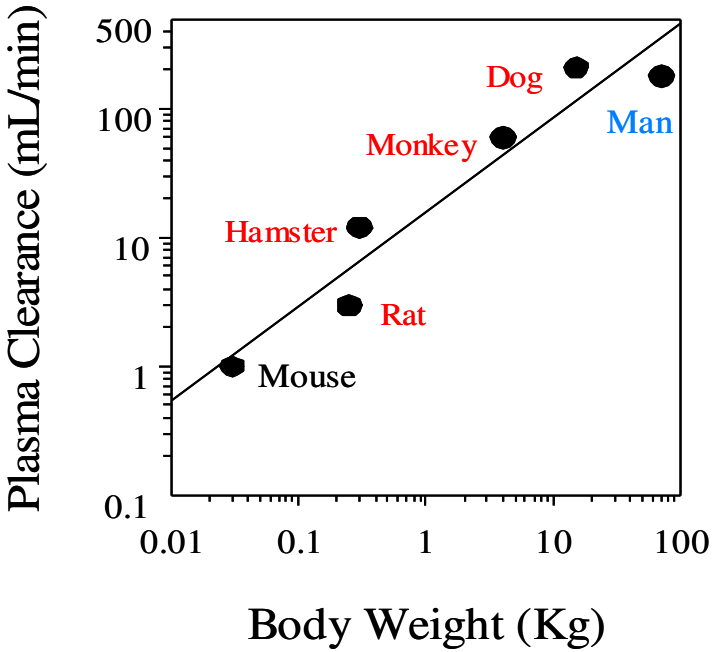
Prediction of human clearance by allometry

Methotrexate (Renal CL)



Passive renal filtration, $\log D_{7.4} \leq 0$

Cyclophosphamide (High CL)



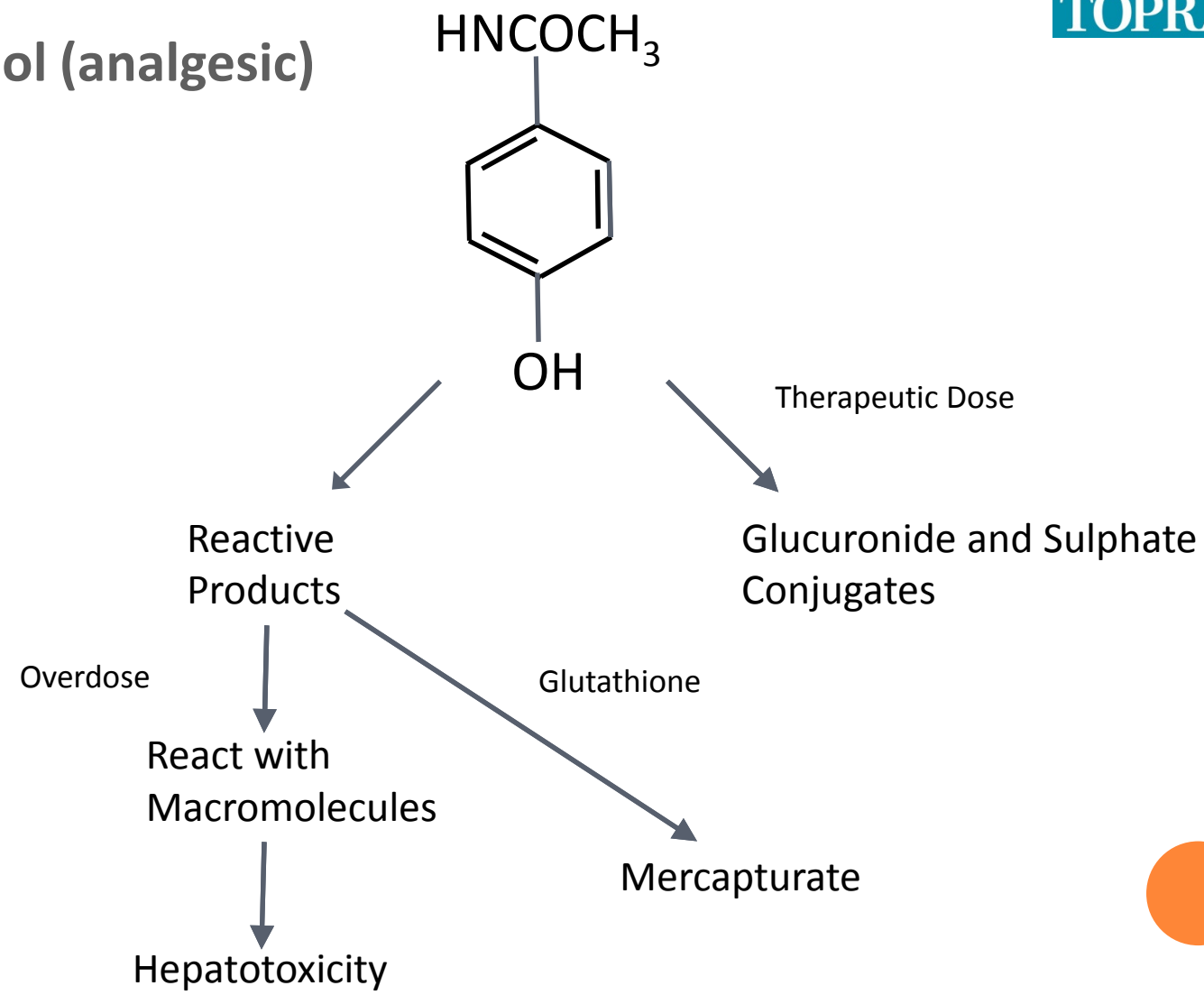
High first pass Cl, low F

Factors Affecting Drug Disposition

Factor	Outcome
Dose	Saturation of absorption and/or elimination
Age	Decreased renal, hepatic and respiratory function
Liver disease	Changes in capacity for drug metabolism
Kidney disease	Decreased elimination of drugs, metabolites and endogenous components Decreased liver function e.g. metabolism
Respiratory disease	Increased liver metabolic capacity
Heart disease/failure	Reduced organ blood flow to all tissues including eliminating organs
Gastro-intestinal disease	Decreased drug absorption
Food and drug interactions	Decreased or increased absorption/bioavailability depending on the drug

Effect of Dose Level

Paracetamol (analgesic)



Effect of Age

Imipramine PK parameters

PK Parameter	Young	Old (>70)
Clearance (ml/min)	950	570
Half-Life (h)	17	30
C_{max} (ng/ml)	10 - 20	40 - 45

Vd and fu unchanged: Decrease in clearance is due to reduced ability of the 'aged' liver to N-dealkylate

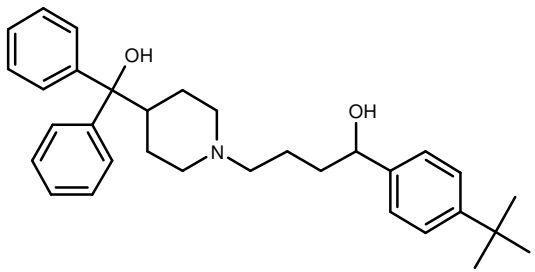
Effects of Disease

- Hepatic disease:
  - Propranolol (high first pass) has increased bioavailability in patients with liver disease ($\downarrow Cl_H$)
  - Decreased metabolism $\rightarrow$ Increased exposure
- Kidney disease:
  - Renal clearance (Cl_R)
  - Decreased excretion $\rightarrow$ Increased hepatic clearance and exposure

Effects of Food and Drug Interactions

- Food effects:
  - No effect
  - Modify absorption/bioavailability e.g. spironolactone (increase); propentophylline (decrease)
- Metabolic Inhibition:
  - Decrease in clearance (↑ t1/2)
    - Cimetidine on diazepam, terfenadine (3A4)
    - Grapefruit juice inhibits CYP3A4: ↑ F % of omeprazole (Losec)
- Metabolic Induction:
  - Increase in clearance (↓ t1/2)
    - Carbamazepine, phenytoin, barbiturates, alcohol (CYP3A4)
    - Cigarettes, BBQ (CYP1A2)

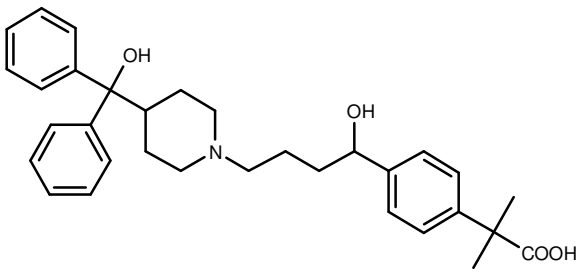
Implications of Terfenadine-Ketoconazole Interaction



Terfenadine Almost complete first pass extraction in man

CYP3A4

Inhibited by Ketoconazole



Active metabolite Responsible for efficacy in man

- High circulating concentrations of terfenadine prolong QT interval of the ECG
- Abnormal heart rhythm
- Small numbers of patients go on to develop fatal Torsade de Pointes
- Terfenadine withdrawn from the market
- Increased questioning of Regulatory Authorities on QT and DDIs

Clinical interaction studies

- Identify metabolising isozyme & potential interacting drugs
  - *In vitro* human liver microsomes with/without known CYP-450 inhibitors/inducers
- Specific studies for potential co-administered drugs
  - Selected based on risk indicated by *in vitro* studies
- Food effects on absorption (Phase I)
- Pharmacokinetic screen (Population Approach in Phase II and III)

When, What and Why for Clinical Pharmacokinetics and Pharmacodynamics?

	Clinical Phase	Pharmacodynamics	Pharmacokinetics
0	Candidate selection	No. Dose used ≤100µg inactive.	Absorption, distribution, clearance, metabolism, half-life
I	Safety Tolerance Activity	Clin obs, ECG, clin chem, haematology, BP Biomarkers *in vivo*/*ex-vivo*	Absorption, clearance, bioavailability, elimination, half-life, PK/PD
II	POC	Safety, Biomarkers, Clinical efficacy outcomes	Absorption, clearance, bioavailability, elimination, half-life, PK/PD
	Dose finding	Safety, Biomarkers, Clinical efficacy outcomes	Absorption, clearance, bioavailability, elimination, half-life, PK/PD
III	Pivotal confirmatory trials	Safety, Clinical efficacy outcomes	Population PK
IV	New formulation New indication	Biomarkers, Clinical efficacy outcomes	Bioequivalence PK/PD for new indication

Use of preclinical and Phase I/IIa data, to support Go/No Go decisions?

Criteria	Go	No Go
PD activity at tolerable doses	Reproducible Relevant Dose/exposure-related	Absent or variable Relevance unproven Not related to dose/exposure
PD duration	Allows dosing regime acceptable to patients	Requires inconvenient, complex dosing regime
PK characteristics	**Linear with dose and time** **Low inter/intra-subject variability**	**Non-linear with dose or time** **High inter/intra-subject variability**
PK/PD relationship	**Well-defined** **Predictable**	**Inadequate** **Unpredictable**
Safety profile	Predictable, Wide therapeutic ratio	Unpredictable Narrow therapeutic ratio
Bioavailability	**Acceptable** **Predictable** **Low inter/intra subject variability**	**Unacceptable** **Unpredictable** **High inter/intra subject variability**
Physico-chemical properties	**Allows adequate exposure in human studies in an appropriate formulation**	**Difficult to achieve adequate exposure in human studies in an appropriate formulation**
Commercial viability	Adequate market size/price profile to achieve return on investment	Market too small/low-priced to ensure adequate return on investment

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