Biopharmaceutics

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Drug Distribution

Introduction

- After absorption, the drug enters into a number of processes called disposition processes
- The phases of drug disposition are:

i. **Distribution-** It is defined as the reversible transfer of drug between blood and other remaining compartments of the body

ii. **Elimination-** It is defined as the irreversible loss of drug from the body. It is further divided into two mechanisms such as biotransformation (metabolism) and excretion

- The free and unbound drug present in the blood stream permeates through the wall of the capillary and enters into the interstitial/ extracellular fluid (ECF)
- The drug present in the ECF permeates through the tissue cells and enters into the intercellular fluid

- This is the rate limiting step, which involves two major factors such as:

i. Rate of perfusion to the extracellular tissue

ii. Permeability of membrane for drugs

- It plays important role in the **onset and intensity** of a pharmacological response as distribution process makes the drug reach the site of action

Characteristics of distribution process

- Distribution is a passive process
- The distribution of drugs in the body depends on their lipophilicity and protein binding
- The extent to which a drug distributes affects the concentration at steady state

- Most drugs exhibit a non-uniform distribution in the body with variations that are largely determined by the ability to pass through membranes and their lipid/water solubility
- Small water soluble molecules and ions diffuse through aqueous channels or pores Lipid – soluble molecules penetrate the membrane itself

Factors affecting distribution of drugs

I. Membrane permeability which depends on

a) Physicochemical properties of drugs

b) Types and characteristics of various physiological barriers

II. Blood perfusion rate

III. Binding of drugs (protein and tissue)

a) Binding of drug to blood components

a) Binding of drugs to extravascular components

IV. Miscellaneous factors

a) Age

b) Pregnancy

c) Obesity

d) Disease states

Physicochemical properties of the drug

- Molecular size

i. Smaller the molecular size of the drug, more easily it crosses the capillary membrane to enter into the ECF

ii. The rate of passive diffusion is inversely proportional to the square root of molecular size

- Degree of ionization

- The un-ionized fraction of a drug is available to cross the cell membrane
- Lipid solubility (partition coefficient)
- Protein binding (free & unbound drug)

Types of barriers

1. Simple endothelial capillary barrier
2. Simple cell membrane barrier
3. Blood brain barrier (BBB)

i. Layer of Glial cells ii. Highly lipophilic drugs

4. Blood cerebrospinal fluid barrier

i. Choroidal plexus cells

5. Placental barrier
6. Blood testis barrier

Blood perfusion rate

- The distribution of drugs is limited in two ways:

1. The drug distribution is permeability rate limited in the case of

A. When the drug is polar, ionic or water soluble

B. The highly selective physiological barriers

2. The drug distribution is perfusion rate limited when

A. The drug is highly lipophilic

B. The membrane is highly permeable

- Perfusion rate is defined as the volume of blood that flows per unit time per unit volume of the tissue

Binding of drugs to Blood components

- Human Serum Albumin (HSA) : Warfarin & Azapropazone, Diazepam, Digitoxin, Tamoxifen binding sites **(Acidic)**
- α_1-acid glycoprotein (AAG) (orosomucoid)

a. Binds with basic drugs eg., Lidocaine, propranolol, imipramine and quinidine

- Lipoproteins

- Globulins

a. Corticosteroid binding-globulin (CBG) or transcortin is highly specific to certain steroids eg., Prednisolone

b. Transcortin also binds thyroxine and vitamin B_{12}

- Erythrocytes

Effect of Plasma Protein Binding

A. Distribution

- Decreases the V_d values

B. Elimination

- Increases elimination/Biological half life

C. Drug effects

- Decreased pharmacological action

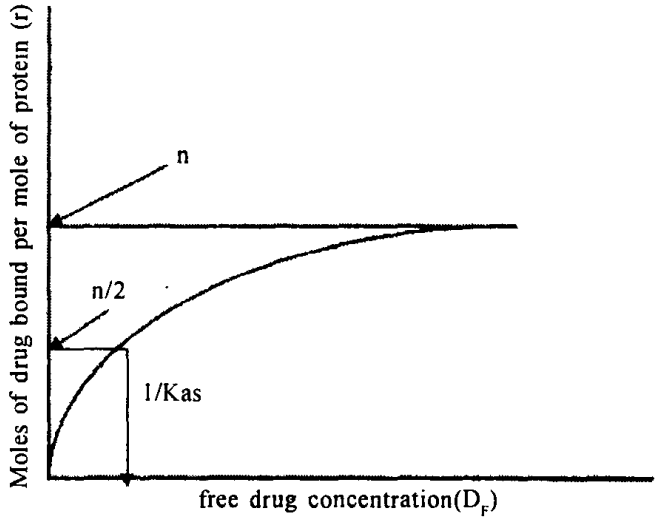
D. Displacement reactions (more significant with highly bound drugs)

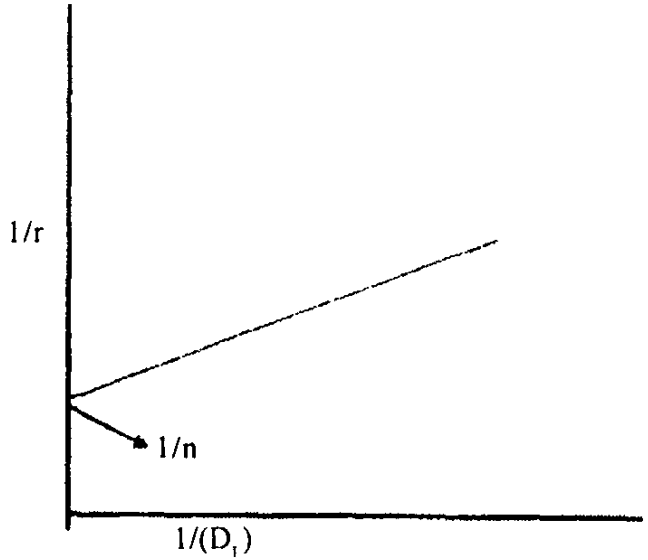
- When a highly protein-bound drug is displaced from its binding site by a second drug or agent, a sharp increase in the free drug in the plasma may occur, leading to toxicity

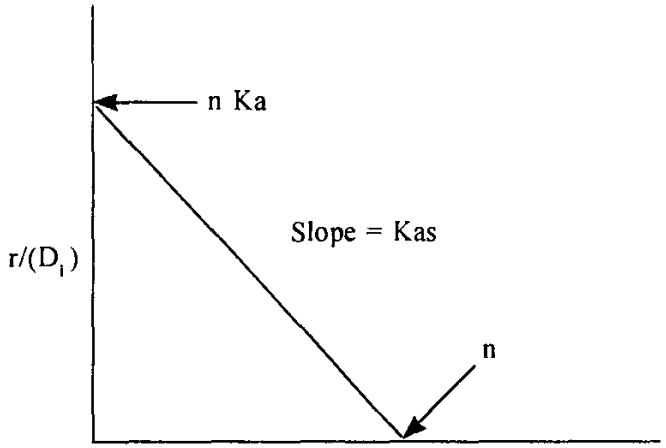
Drug	Before displacement	After displacement	Percent increase in free drug
Oxazepam			
Percent bound	95	90	
Percent unbound	5	10	+100
Phenobarbital			
Percent bound	55	50	
Percent unbound	45	50	+10

Methods to determine Protein Binding

1. Direct plot method
2. Double reciprocal plot method
3. **Scatchard Plot method**







Apparent volume of Distribution (V_d)

- It is the hypothetical volume of body fluid into which drug gets distributed
- It can be calculated by:

$$V_d = \frac{\text{Amount of drug in body at equilibrium}}{\text{Plasma drug concentration}}$$

- More the protein binding, less will be the V_d
- More the tissue binding, more will be the V_d

- The volume of distribution is a function of four major factors:

1. The size of the organ into which the drug distributes
2. The partition coefficient of the drug between the organ and circulating blood
3. The blood flow to the distributing organs and
4. The extent of binding of the drug both in blood and in various tissues

Tracer/ Markers for estimation of body fluids

- Plasma volume – Evans Blue
- ECF – Isotopes of Chloride, Bromide, Inulin, Mannitol
- Total Body water – D_2O, Antipyrine, Radio labelled Chromium

Time to recap

1. Apparent volume of distribution increases when

A. More tissue binding of drug

B. More protein binding of drug

C. Both

D. None

Ans is **A**

2. If a drug has very small volume of distribution (V_d) it is likely that this drug

A. Has a short biological life
B. Does not accumulate in various and organs
C. Is not bioavailable
D. Will not be effective

Ans is **B**

3. What effect dose plasma protein binding have on bio transformation?

A. Change the mechanism
B. Increases the formation of metabolites
C. Slows the process
D. Has no effect

Ans is C

4. Which statement is correct?

A. Mainly acidic drug binds to albumin
B. Mainly basic drug binds to albumin
C. Acidic drug binds only to α-acid glycoprotein
D. None of the above

Ans is **A**

5. The initial distribution of a drug into tissue is determined chiefly by

A. Rate of blood flow to the tissue
B. Plasma protein binding of the drug
C. Affinity for the tissue
D. Stomach emptying tissue

Ans is **A**

6. Which of the following junctions is responsible for low distribution of drug to cerebrospinal fluid from blood

A. Choroidal cell junction
B. Glial cell junction
C. Basement cell junction
D. Both A and B

Ans is **A**

7. Which of the following approaches have been used to promote crossing the BBB by drug?

A. Use of permeation enhancers such as DMSO
B. Osmotic disruption of BBB by mannitol
C. Use of dihydropyridine redox system
D. All of above

Ans is **D**

8. Which of the following barrier is responsible for transfer of nutrients from mother to foetus?

A. Simple cell membrane barrier
B. Blood–brain barrier
C. Blood placental barrier
D. Blood–cerebrospinal fluid barrier

Ans is **C**

9. Which one of the following marker is used to measure total body water

A. Antipyrine
B. Evans blue
C. Na^+
D. Mannitol

Ans is **A**

10. Inulin is used as a marker for measurement of

A. Plasma
B. ECF
C. ICF
D. Total body water

Ans is **B**

11. Which of the following binding site is not available in HSA?

A. Azapropazone
B. Diazepam
C. Digitoxin
D. Phenytoin

Ans is **D**

Elimination of Drugs

- Drug elimination means irreversible removal of the drug from the body by all possible routes
- Elimination occurs by excretion and metabolism
- Metabolism is the process by which the drug is chemically modified in the body and the end product of this modification is called metabolite

- Liver is the major organ for metabolism
- Drug excretion refers to the removal of unchanged drug (intact drug)
- Kidney is the major organ for excretion

1. Metabolism (Biotransformation)

- The pathways of metabolism may be:

1. Phase I reactions

a) Oxidation

i. Microsomal

ii. Non microsomal

a) Reduction

- Azo, Nitro etc

a) Hydrolysis

- Ester, amide, hydrazine

a) Hydration

1. Phase II reactions

- Glucuronide conjugation
- Sulfation
- Methylation
- Acetylation
- Amino acid conjugation
- Glutathione conjugation

Enzyme Inducers

Classification	Examples
Analgesics	Nikethamide
Antipyretic and Analgesic	Antipyrine
Anti-inflammatory	Phenylbutazone
Antibiotics	Rifampicin
Anticonvulsants	Carbamazepine
Antifungal drugs	Griseofulvin
Antimalarials	Quinine
Sedatives and hypnotics	Phenobarbitone
Diuretics	Spiranolactone

Enzyme Inhibitors

Drugs	Xenobiotics
Allobarbital	Ethylene
Secobarbital	Acetylene
Amphetamine	Piperonal
Cimetidine	Safrole
Isoniazid	Peperonylbutoxide
Dapsone	Carbontetrachloride
Fenfluramine	Parathion
Sulfanilamide	7,8-benzoflavone
Chloramphenicol	
Amantadine	
Indomethacin	
MAOinhibitors(Isocarboxazid, Phenelzine)	
Xanthine oxidase inhibitors (Allopurinol)	

II. Excretion of Drugs

- Pathways of excretion

i. Renal excretion (Major)

ii. Salivary

iii. Biliary

iv. Hepatic

v. Secretion of drug into milk

vi. GI excretion

vii. Genital excretion

viii. Excretion through sweat

Renal excretion

1. **Glomerular filtration** is a passive process by which small molecules and drugs are filtered through the glomerulus of the nephron

- Drugs bound to plasma proteins are too large to be filtered at the glomerulus
- Drugs such as **creatinine and inulin** are not actively secreted or reabsorbed. They are used to measure the **glomerular filtration rate (GFR)**

2. **Tubular reabsorption** is a passive process that follows Fick's law of diffusion

i. Lipid-soluble drugs are reabsorbed from the lumen of the nephron back into the systemic circulation

ii. For weak electrolyte drugs, urine pH affects the ratio of nonionized and ionized drug

- If the drug exists primarily in the nonionized or lipid-soluble form, then it is reabsorbed more easily from the lumen of the nephron
- If the drug exists primarily in the ionized or water-soluble form, then it is excreted more easily in the urine
- Alteration of urine pH alters the ratio of ionized to nonionized drug and affects the rate of drug excretion. Eg., Alkalinization of the urine increases the excretion of weak acids (Salicylates)

iii. An increase in urine flow caused by simultaneous administration of a **diuretic** decreases the time for drug reabsorption. Consequently, more drug is excreted if given with a diuretic

- Exceptionally, some drugs undergo active tubular reabsorption Eg., Glucose and Vit B_{12}

3. **Active tubular secretion** is a carrier-mediated active transport system that requires energy

- Shows competition effects. Eg., **probenecid** (a weak acid) competes for the same system as penicillin, decreasing the rate of penicillin excretion, resulting in a longer penicillin $t_{1/2}$
- The renal clearance of drugs that are actively secreted, such as ***p*-aminohippurate (PAH)**, is used to measure **effective renal blood flow (ERBF)**

- The renal tubule membranes favor the transport of lipid-soluble drugs
- Drugs that are poorly lipid-soluble or ionized are poorly reabsorbed
- The reabsorption of weak acidic or weak basic drugs is influenced by the pKa of a drug and the pH of the fluid in the renal tubule (i.e., urine pH)

- The parameter which explain the excretion is "Renal clearance"
- It can be estimated by:

$$Cl_R = \frac{dx_u / dt}{C}$$

dx_u/dt= instantaneous rate of urinary excretion of the drug

- Clearance can also be estimated by:

$$\mathbf{Cl_R = \frac{(filtration\ rate + secretion\ rate - reabsorption)}{plasma\ drug\ concentration}}$$

- Renal Clearance ratio can be estimated by:

$$\mathbf{Renal\ clearance\ ratio = \frac{renal\ clearance\ of\ drug}{renal\ clearance\ of\ creatinine\ or\ inulin}}$$

Bioavailability & Bioequivalence

Introduction and Definitions

- After extravascular administration, the drug has to reach the systemic circulation before it can be distributed to all parts of the body

- The amount of the drug administered may/may not be equal to the amount of the drug that reaches the systemic circulation after extra vascular administration

- The fraction of the administered dose that reaches the systemic circulation is the drug bioavailability

1. Bioavailability

a. Absolute Bioavailability

- It is the fraction of the administered dose that reaches the systemic circulation
- It is determined by comparing the amount of the drug that reaches the systemic circulation after extravascular administration and after IV administration of the same drug
- It can take values between 0 and 1
- It does not have any units and can be expressed as percent

b. Relative Bioavailability

- The bioavailability of the drug product relative to the bioavailability of a second drug product that contains the active drug moiety
- Determined by comparing the amounts of the drug that reach the systemic circulation after administration of an oral drug product and an oral reference standard preparation of the same active drug moiety
- It can take any value above 0. It can be more than one because the dosage form under study may have higher bioavailability than the reference standard formulation

- **Bioavailability**
- The rate and extent of the unchanged drug that reaches the systemic circulation (Regulatory)
- **Bioequivalence**
- Two drug products are considered BIOEQUIVALENT

a. If there is no statistical significant difference between the rate and extent of test (generic) and reference product (RLD)

b. If both products are pharmaceutical equivalents

c. If administered in the same molar dose of the active ingredient in the same chemical form in a similar dosage form, by the same route of administration, and under the same experimental conditions

- **Causes for incomplete Bioavailability**

1. Route of administration
- Eg Oral, IV, IM etc
2. Factors related to formulation
- Various excipients used and type of dosage form
3. Factors related to drug
- Various physicochemical factors like Solubility, PS, Crystal type, lipophilicity etc
4. Factors related to patients
- Various physiological factors and disease states

- **Role of Bioavailability studies**

- To evaluate the absolute systemic availability of an oral, topical, intramuscular, or any other dosage form
- To determine if bioavailability parameters are linear over the proposed clinical dose range
- To estimate the inter and intra subject variability
- To study food effects
- To define the effect of changes in the physicochemical properties of the drug substance and the effect of the drug product (dosage form) on the pharmacokinetics of the drug

- **Role of Bioequivalence studies**

1. Useful during IND or NDA development phase to establish link between

a. Early and late clinical trial formulations

b. Formulations used in clinical trial and stability studies, if they are different

c. Clinical trial formulations and to-be-marketed drug product

2. Critical component of ANDA submissions

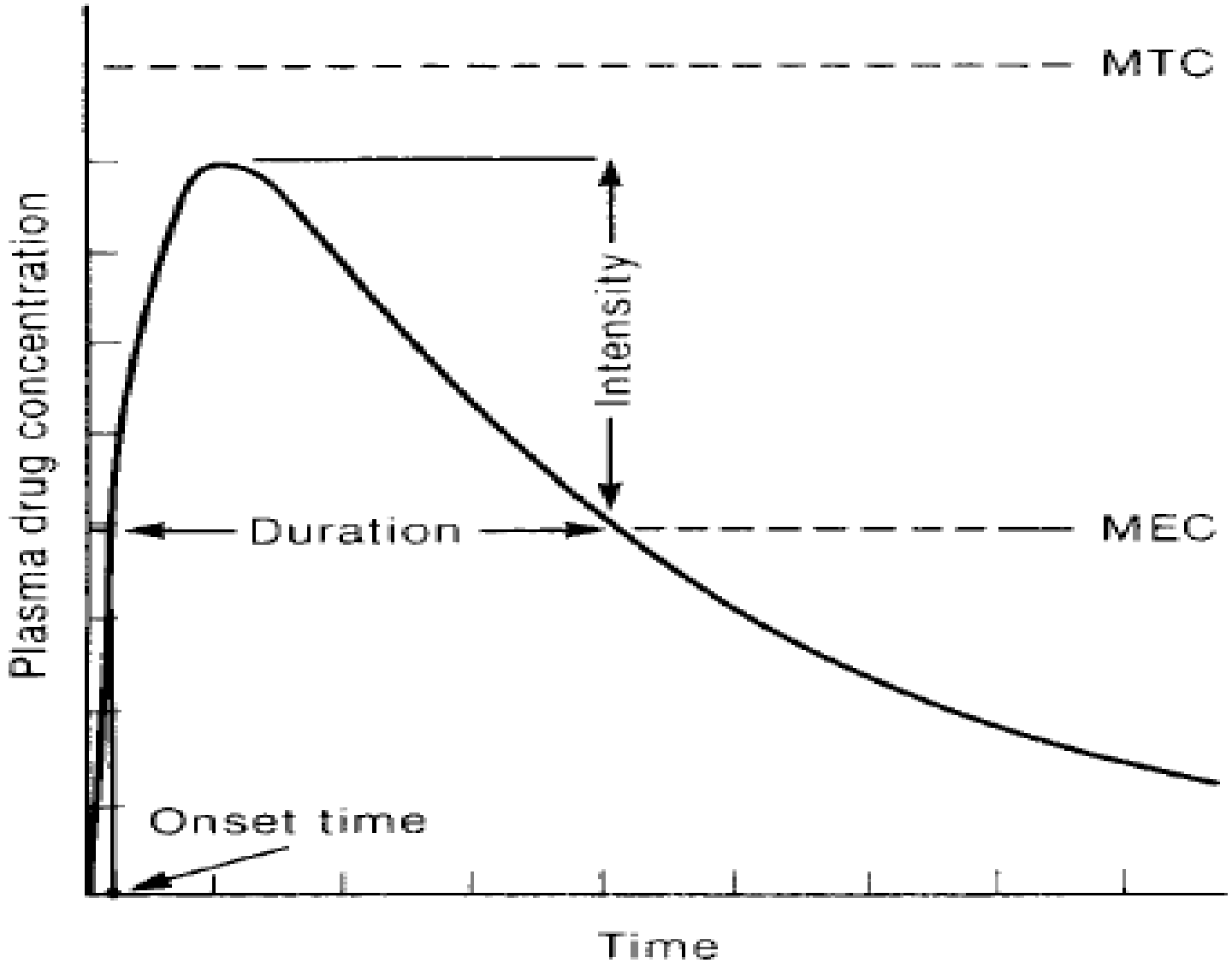
- To demonstrate bioequivalence between a pharmaceutically equivalent generic drug product and the corresponding reference listed drug (switchability)
- Establishing bioequivalence allows a regulatory conclusion of therapeutic equivalence

3. Scale-up and post-approval changes (SUPACs)

Methods to assess Bioavailability

1. Acute Pharmacodynamic Methods

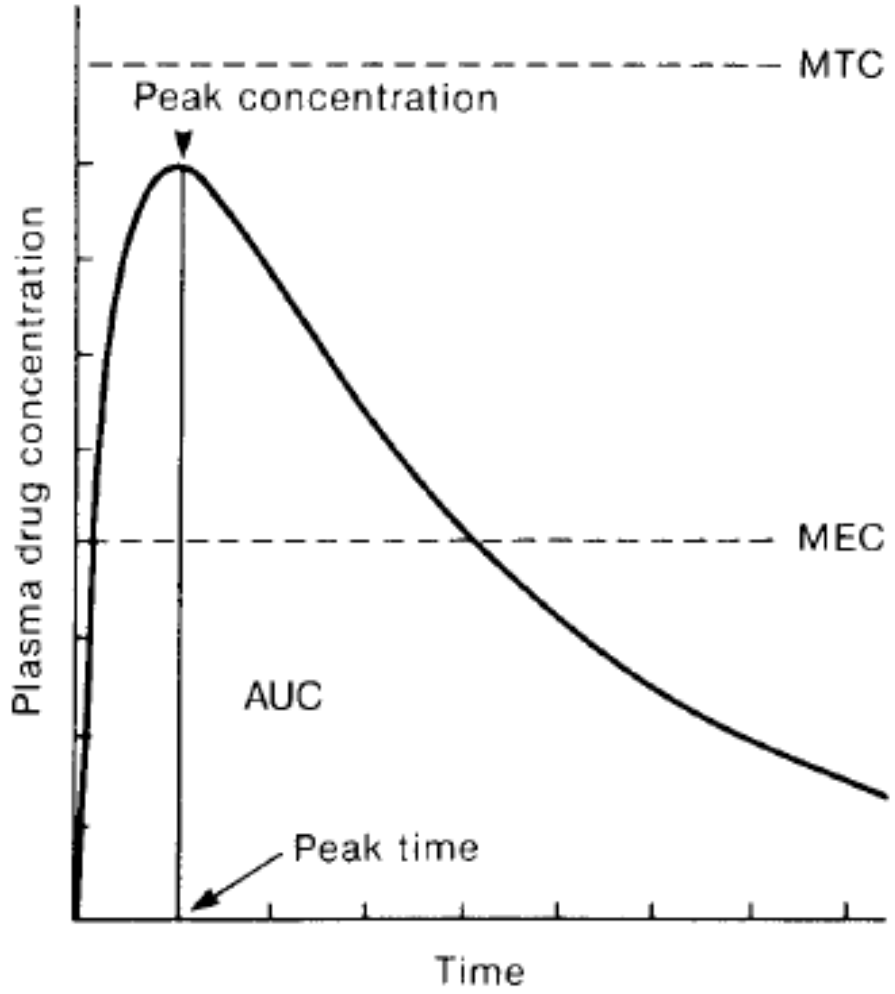
- Changes in heart rate, blood pressure, electrocardiogram (ECG), clotting time, or forced expiratory volume in 1 sec (FEV1)
- Quantitation of the pharmacological effect versus time profile can be used as a measure of bioavailability and/or bioequivalence



Parameters measured are:

1. Onset time
2. Duration of action
3. Intensity of action
4. Therapeutic window

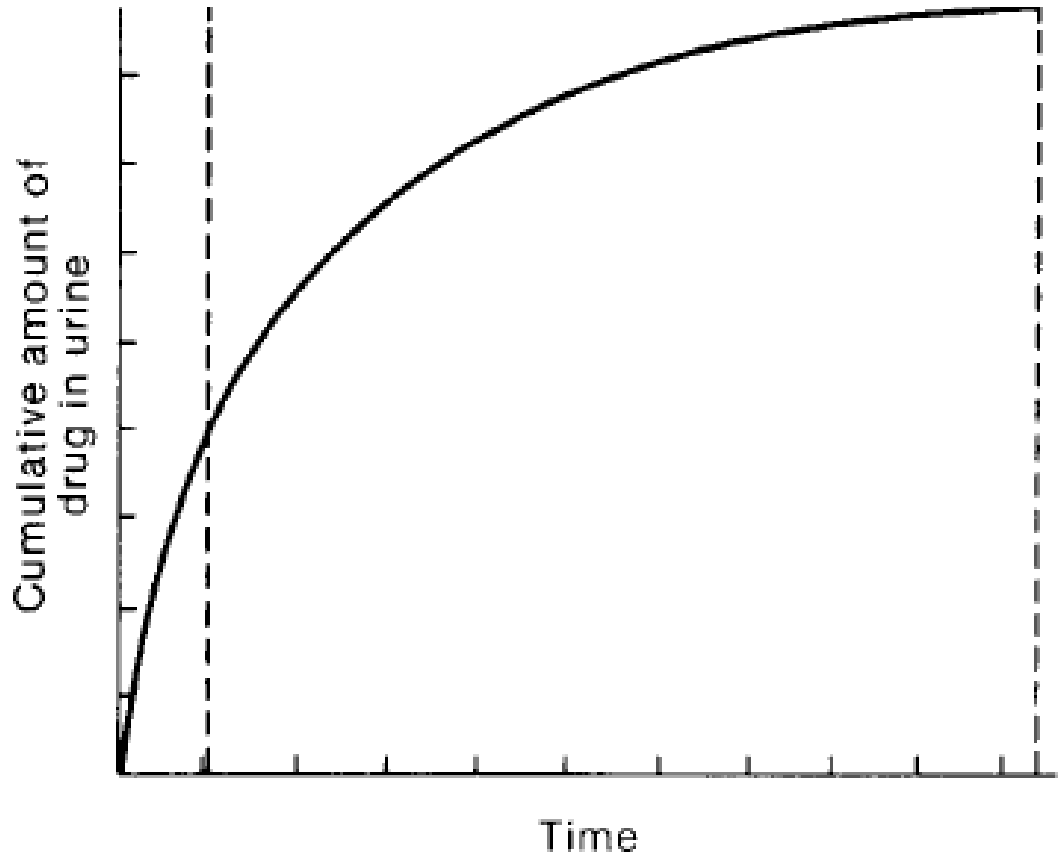
2. Plasma drug concentration



Parameters measured are:

1. Time for plasma peak concentration (t_{max})
2. Peak plasma drug concentration (C_{max})
3. Area under curve (AUC)

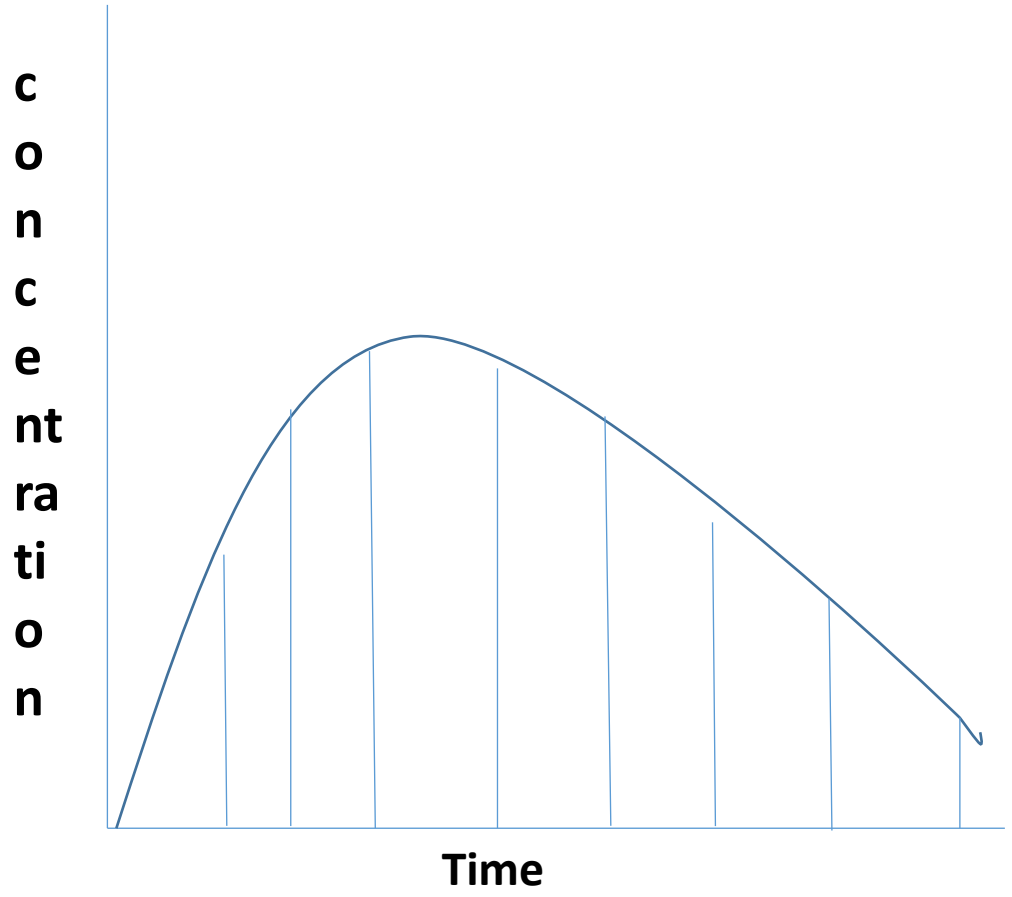
3. Urinary drug excretion



Parameters measured are:

1. Cumulative amount of drug excreted unchanged in urine
2. Rate of drug excretion urine
3. Time for the drug to completely excreted

- **Calculation of AUC**
- **Trapezoidal Rule**
- Divide conc vs time profile into different trapezoids
- Calculate the area of each trapezoid
- AUC can be calculated by adding individual areas of all trapezoids and adding terminal area



- **Formula to calculate Absolute Bioavailability**

$$\text{Absolute Bioavailability} = \frac{\text{AUC}_{\text{oral}}}{\text{AUC}_{\text{IV}}}$$

- **Formula to calculate Relative Bioavailability**

$$\text{Relative Bioavailability} = \frac{\text{AUC}_{\text{test}}}{\text{AUC}_{\text{Std}}}$$

- **Formula to calculate Absolute Bioavailability**

$$\text{Absolute Bioavailability} = \frac{\text{AUC}_{\text{oral}}}{\text{AUC}_{\text{IV}}} \times \frac{\text{Dose}_{\text{iv}}}{\text{Dose}_{\text{oral}}}$$

- **Formula to calculate Relative Bioavailability**

$$\text{Relative Bioavailability} = \frac{\text{AUC}_{\text{test}}}{\text{AUC}_{\text{Std}}} \times \frac{\text{Dose}_{\text{std}}}{\text{Dose}_{\text{test}}}$$

Bioavailability/Bioequivalence Study Protocol

A. Study objective

B. Study design

1. Experimental design

2. Washout period

3. Drug products

4. Route of administration

5. Dosage regimen

6. Frequency and duration sampling

7. Randomization of drug administration

8. Single versus multiple dose study design

9. Subjects

a. Healthy subjects versus patients

b. Subject selection

c. Study conditions

10. Analysis of biological fluids

C. Methods of assessing bioavailability

1. Plasma data

2. Urine data

3. Acute pharmacological effect

4. Clinical response

D. Analysis and presentation of data

1. Statistical treatment of data, ANOVA

2. Format of data

Statistical evaluation of the data

- The statistical methodology is called the *two one-sided test procedures*
- Two situations are tested with this statistical methodology

1. Whether a generic product (test), when substituted for a brand-name product (reference), is significantly less bioavailable
2. Whether a brand-name product (reference), when substituted for a generic product (test), is significantly less bioavailable

- A difference of **> 20%** for each of the aforementioned tests was determined to be significant

- An analysis of variance (ANOVA) should be performed on the log transformed AUC and C_{max} values obtained from each subject
- The 90% confidence intervals for both pharmacokinetic parameters, AUC and C_{max} must be entirely within the 80% to 125% boundaries based on log transformation of the data
- The ratio of the means of the study data (test to reference) should lie in the center of the 90% confidence interval, or close to 100%

Waiver of an in vivo bioequivalence study (Biowaiver)

- *In vitro* dissolution (drug-release) study between the test and the reference products

1. Drug products given as a solution such as oral, parenteral, ophthalmic, or other solutions because bioequivalence is self-evident

2. IR solid oral drug products that meet biopharmaceutics classification system (BCS) class 1
3. Drug products containing a lower dose strength. (The drug product must be in the same dosage form, lower strength, and is proportionately similar in its active and inactive ingredients)

In vitro In vivo Correlation (IVIVC)

- **FDA**
- "A predictive mathematical model describing relationship between in-vitro property of a dosage form and in-vivo response"
- ***In vitro*** **properties** are rate or extent of drug released under a given set of conditions
- ***In vivo*** **properties** are plasma drug concentration or amount of drug absorbed expressed in terms of C_{max}, AUC, etc

Levels of Correlation

Level A correlation

- Predictive model for the point-to-point relationship between the entire *in vitro* release time course and entire *in vivo* response time course
- Level A correlation is the most informative and very useful from a regulatory perspective

Level B correlation

- A predictive mathematical model for relationship between summary parameters that characterize the in-vitro and in-vivo time course
- It compares

1. MDT vitro to MDT vivo, or
2. MDT vitro to MRT, or

3. In-vitro Dissolution Rate Constant (kd) to Absorption Rate Constant (ka)

- Comparison using **Statistical Moment Analytical Method**
- This is of limited interest and least useful for regulatory purposes because more than one kind of plasma curve produces similar mean residence time

Level C correlation

- It relates **one dissolution time point** (**$t_{50\%}$, $t_{90\%}$** , dissolution observed at 1 h, etc.) to one mean pharmacokinetic parameter such as AUC, T_{max} or C_{max}
- Level C correlations can be useful in the early stages of formulation development when pilot formulations are being selected

Multiple level C correlation

- It relates one or more pharmacokinetic parameters to the percent drug dissolved at several time points of dissolution profile and thus may be more useful
- If a Multiple Level C correlation is possible, then a Level A correlation is also likely and is preferred.

Various Parameters Used in IVIVC Depending on the Level

Level	In vitro	In vivo
A	Dissolution curve	Input (absorption) curves
B	Statistical Moments: MDT	Statistical Moments: MRT, MAT
C	Disintegration time, Time to have 10, 50, 90% Dissolved, Dissolution rate, Dissolution efficiency	C_{max}, T_{max}, K_a, Time to have 10, 50, 90% absorbed, AUC (total or cumulative)

Time to recap

1. The useful variable from in vitro dissolution test data for IVIVC includes:

A. Sampling interval
B. Sample volume
C. Volume of dissolution fluid
D. None of above

Ans is **D**

2. When Statistical Moment theory is used for IVIVC

A. Mean dissolution time is correlated with mean residence time
B. LD_{50} is correlated with rate constant of dissolution
C. Drug excreted unchanged in urine is correlated with percent drug dissolved
D. 63.2% of drug dissolved is correlated with 63.2% of initial concentration reduction

Ans is **A**

3. Which one of the following process is responsible for higher half-life of drug?

A. Drug absorption
B. Excretion
C. Metabolism
D. Protein binding

Ans is **D**

4. Barbiturate excretion in urine may be increased by

A. Dialysis
B. Acidification
C. Alkalization
D. None of above

Ans is C

5. Zolmitryptan is given i.v to the patient in dose of 1.2 mg/kg (AUC = 450 µg.hour/L).Same drug was given as oral SR tablet in dose 8.0 mg/kg (AUC = 1040 µg.hour/L). What is the absolute bioavailability of SR tablet?

..\Bioavailability Calculation.jpeg

A. 30%

B. 35%

C. 38%

D. 42%

Ans is **B**

6. For two drug products, generic and brand to be considered bio-equivalent, the 90% confidence intervals about the ratio of the means of the C_{max} and AUC values for generic/brand product must be within

A. 80%–125% of the brand product
B. 80%–100% of the brand product
C. 80%–85% of the brand product
D. 80%–90% of the brand product

Ans is **A**