B.Pharmacy
Subject-Biopharmaceutics and pharmacokinetics
Subject Code-BP604T

Module-2

BIOTRANSFORMATION

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Objective of course:

To understand the concept of biotransformation,bioavailability and bioequivalence of drug products and their significance.

Learning Outcomes:

- Students will learn about the basic metabolic pathways and factors effection metabolism of drugs.
- Students will learn about the various types and methods to measure bioavailability,IVIVC.

OVERVIEW

- Definition
- Consequences
- Types
- Phase I/II in detail
- Enzyme Induction/Inhibition
- First Pass Metabolism

INTRODUCTION

- **Xenobiotics**- substances foreign to body
- include- Drugs, Processed food, Food additives, Cosmetic products, Environmental pollutants, Agrochemicals,
- Phytoallexins (dietary plant toxins)

Biotransformation needed for detoxification& protect the body from ingested toxins.

Definition

- Chemical alteration of drug in the body.
- ***Non polar lipid soluble*** compounds are made ***polar lipid insoluble***, so that they are easily excreted.
- Drugs which do not undergo biotransformation – Streptomycin, neostigmine….(highly polar drugs)
- **SITES**

  Primary site – Liver

  Others – Kidney, Intestine, Lungs, Plasma…

Drug Biotransformation –

convert lipophilic / hydrophobic drug (to enter cells) to hydrophilic metabolites.

Advantages

- Termination of drug action - (↓ toxicity)
- Reduced lipophilicity.
- Renal / biliary excretion ↑ - (↓renal reabs)

Absorbed drugs – 3 changes

- Metabolic changes by Enzymes

  (Microsomal, Cytoplasmic, Mitochondrial)

- Spontaneous Molecular rearrangement – **HOFMANN ELIMINATION**

- Excreted unchanged (highly polar drugs) -

  Aminoglycosides,Methotrexate,Neostigmine

CONSEQUENCES

- **A) Drug inactivation -** inactive or less active Propranolol,Pentobarbitone,Morphine, Chloramphenicol,Paracetamol,Ibuprofen, lignocaine

- **B) Active drug to Active metabolite-** active metabolite effect is due to parent drug and its active metabolite

C) Inactive drug (Prodrug) - Active drug

Prodrugs are inactive drugs which need BT in the body to form active metabolites.

- **ADV**
- More stable
- Better BA
- Less toxicity

Levodopa	-	Dopamine
Enalapril	-	Enalaprilat
αMethyl dopa	-	αMethyl Norepinephrine
Dipivefrine	-	Epinephrine

Proguanil	-	Proguanil triazine
Prednisone	-	Prednisolone
Bacampicillin	-	Ampicillin
Sulfasalazine	-	5amino salicylic acid
Cyclophosphamide	-	Aldophosphamide
Mercaptopurine	-	Methyl Mercaptopurine
Prontosil	-	Sulfanilamide
Acyclovir	-	Acyclovir triphosphate

TYPES

BIOTRANSFORMATION REACTIONS - 2 TYPES

- **Phase I / Non synthetic / Functionalization**
  - A functional group is generated
  - Metabolite – active or inactive

- **Phase II / Synthetic / Conjugation**
- An endogenous radical is conjugated
- Metabolite is usually inactive

Phase I Reactions

- Oxidation
- Reduction
- Hydrolysis
- Cyclization
- Decyclization

Phase II Reactions

- Glucuronide conjugation
- Acetylation
- Methylation
- Sulfate conjugation
- Glycine conjugation
- Glutathione conjugation
- Ribonucleotide / Ribonucleoside synthesis

PHASE I REACTIONS

a) OXIDATION

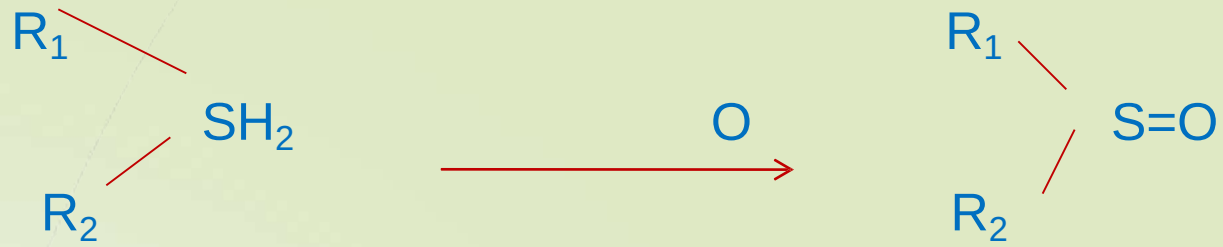
- Addition of Oxygen / negatively charged radical or removal of Hydrogen / Positively charged radical
- Oxidation is the main process of metabolism
- Produces unstable intermediates - Epoxides, Superoxides, Quinones

- Oxidation – 9 types

1.OXIDATION AT NITROGEN ATOM

- Chlorpheniramine
- Dapsone
- Meperidine

2.OXIDATION AT SULPHUR ATOM



- Chlorpromazine
- Chloramphenicol

3.ALIPHATIC HYDROXYLATION

- Hydroxyl group added to drug

$$RCH_2CH_3 \xrightarrow{O} RCHOHCH_3$$

- Salicylic acid to Gentisic acid
- Ibuprofen
- Tolbutamide, Chlorpropamide,

4.AROMATIC HYDROXYLATION

- Phenytoin
- Phenobarbitone
- Propranolol

5.DEALKYLATON AT OXYGEN ATOM

- Phenacetin to Paracetamol

6.DEALKYLATON AT NITROGEN ATOM

- Amitriptyline to Nortriptyline

7.DEALKYLATON AT SULPHUR ATOM

$$RSCH_3 \xrightarrow{O} RSH + CH_2O$$

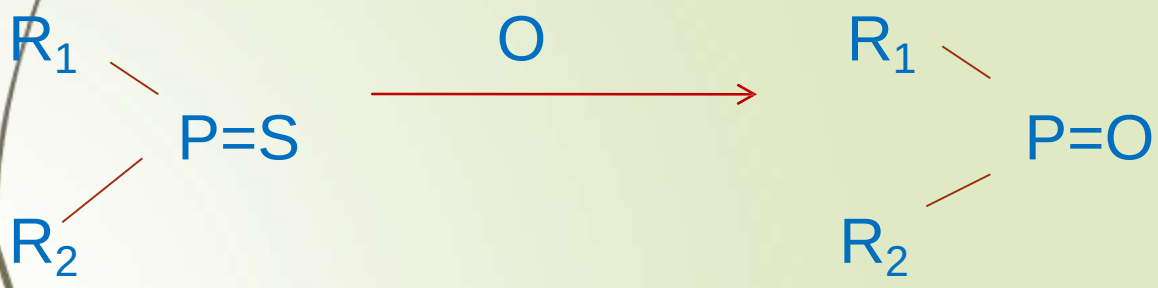
- 6Methyl thiopurine to Mercaptopurine

8.OXIDATIVE DEAMINATION

$$RCHNH_2\,R \xrightarrow{O} RCOR + NH$$

- Amphetamine

9.DESULFURATION



- Parathion to Paraoxon

Main enzymes are the Oxygenases -

- **MICROSOMAL MONOOXYGENASES** in liver (Cytochrome p450/CYP)- drugs

CYP(450)s require NADPH & Oxygen

Drug Metabolizing Enzymes – 2 types

- Microsomal – CYP 450, UDPGT
- Non microsomal – Flavoprotein oxidases,esterases…

NONMICROSOMAL OXIDATION

- Mitochondrial enzymes -**MAO**—Oxidative deamination of Adrenaline,5HT,Tyramine

- Cytoplasmic enzymes - **Dehydrogenases**-

  Alcohol oxidation to Acetaldehyde & Acetic acid

- Plasma oxidative enzymes- **Histaminase, Xanthine oxidase**

b) REDUCTION

- Addition of Hydrogen / positively charged radical or removal of Oxygen / negatively charged radical

MICROSOMAL REDUCTION by Monooxygenases need NADPH & cytochrome c reductase.

A.**NITRO Reduction-** $RNo_2 \longrightarrow RNH_2$

- Chloramphenicol to aryl amine metabolite

B.**KETO Reduction** - $R\text{-}\overset{\overset{O}{\|}}{C}\text{-}R_1 \longrightarrow R\text{-}\overset{\overset{OH}{|}}{C}H\text{-}R_1$

- Cortisone to Hydrocortisone,

C. **AZO Reduction**

- Prontosil to Sulfanilamide

NON MICROSOMAL REDUCTION

- Chloral hydrate to Trichloro ethanol,

c) HYDROLYSIS

- Drug is split combining with water
- Ester + water $\xrightarrow{\text{Esterases}}$ Alcohol & Acid
- Microsomal hydrolysis

  Pethidine to meperidinic acid

- Non microsomal hydrolysis – Esterases,Amidases & Peptidases

  Atropine to Tropic acid

d) CYCLIZATION

- Formation of ring structure from a straight chain compound. Eg: Proguanil

e) DE CYCLIZATION

- Ring structure opened
- Phenytoin, Barbiturates

PHASE II REACTIONS CONJUGATION / TRANSFER

- Drug / phase I metabolite combines with endogenous substance derived from carbohydrates/ proteins.
- covalent bond formation between functional group of drug & endogenous substrate
- Endogenous-Glucuronic acid,Amino acids, Sulfates,Acetates,Glutathione
- Represent terminal inactivation – True detoxification reactions.

- Conjugates-
  - hydrophilic
  - ionized,
  - ↑mol.weight,
  - inactive
- Excreted in urine/ bile/ faeces.
- Phase II- need energy
- 7 types of reactions

1.CONJUGATION WITH GLUCURONIC ACID

- UDP glucuronyl transferases
- Conjugates with OH & COOH are conjugated with glucuronic acid derived from glucose

Drug + UDPGA $\xrightarrow{\text{Microsomal Glucuronyl transferase}}$ Drug glucuronide + UDP

- Drugs - Aspirin,Paracetamol,PABA, Metronidazole,Morphine, Diazepam

- ↑Mol.weight – favours biliary excretion
- Drug glucuronides excreted in bile are hydrolyzed by intestinal microfloral enzymes - parent drug released - reabsorbed into systemic circulation- ↓excretion ↑duration of action

  - Oral contraceptives, Phenolphthalein

- Endogenous substrates - Steroid,Thyroxine,Bilirubin

2. ACETYLATION

- Drugs with Amino or Hydrazine groups - INH,PAS,Hydralazine,Sulfonamides Procainamide,Dapsone. (Code - **SHIP**)

- $R\text{-}NH_2$ —(N Acetyl transferase; Acetyl CoA)→ $R\text{-}NHCOCH_3$

- Genetic polymorphism
- Acetylation- Rapid / Slow

3. CONJUGATION WITH SULFATE

- Drug groups-Amino, Hydroxyl
- Cytoplasmic Enzymes - Sulfotransferases / Sulfokinases.
- Methyl dopa, Steroids,

  Chloramphenicol, Warfarin

4. CONJUGATION WITH GLYCINE

- Drug group – Carboxylic acid
- Salicylic acid , Benzoic acid

5. CONJUGATION WITH GLUTATHIONE

- Drug groups-Epoxide, Quinone
- Toxic metabolites of Paracetamol, Ethacrynic acid
- Cytoplasmic Enzyme - Glutathione S- Transferase

6. METHYLATION

- Drugs with Amino & Phenol groups
- Histamine, Adrenaline, Nicotinic acid, Dopamine, Methyl dopa, Captopril
- Enzyme- Methyl transferase
- Endogenous substance- Cysteine, Methionine

7. RIBONUCLEOTIDE /RIBONUCLEOSIDE SYNTHESIS

- Action of Purine & Pyrimidine antimetabolites
- 6 Mercaptopurine

INHIBITION OF DRUG METABOLISM

- One drug can inhibit the metabolism of another drug
- ↑ in circulating levels of slowly metabolised drug
- Prolongation or potentiation of its effects

- **Consequences**
  - Precipitate toxicity of the object drug.
  - can be therapeutically beneficial. Eg: aversion of alcohol with disulfiram, Reversal of SKM paralysis of d-tc by neostigmine.

- **V**alproate
- **K**etoconazole
- **C**imetidine
- **C**iprofloxacin
- **E**rythromycin
- **INH**

- **Code – V**itamin **K c**annot **c**ause **e**nzyme **i**nhibition**.**

MICROSOMAL ENZYME INDUCTION

- Drugs, insecticides, carcinogens will induce the synthesis of microsomal enzyme proteins
- Accelerated metabolism and reduced pharmacological response

- Consequences
  - Drug- drug interactions
  - Can lead to toxicity. Eg: Alcoholics more prone to hepatotoxicity of paracetamol due to↑ production of NABQI , Pptn of a/c intermittent porphyria by barbiturates.

- Therapeutic benefit. Eg: To treat neonatal jaundice
- Decreased duration of action. Eg: OCP failure

- **G**riseofulvin
- **P**henytoin, Primidone
- **R**ifampicin
- **S**moking
- **C**arbamazepine
- **Ph**enobarbitone

- **Code - GPRS Cell Phone**

First Pass Metabolism

- Presystemic metabolism/ First pass effect
- *Metabolism of a drug during its passage from the site of absorption into the systemic circulation*.
- ↓ed BA
- ↓ed therapeutic response
- **SITES**
  - Gut wall
  - Gut lumen
  - Liver (major site)
  - Lungs
  - Skin

Attributes of drugs with FPM

- Oral dose is higher than sublingual or parenteral.
- Marked individual variation in oral dose – difference in extent of FPM.
- Oral BA is increased in patents with severe liver disease.
- Drugs with FPM usually have short plasma $t_{1/2}$.
- Oral BA is increased if another drug competing with it in first pass metabolism is given concurrently. Eg: CPZ & Propranolol

Bioavailability and Bioequivalence Studies

Bioavailability

Measurement of the relative amount & rate at which,
the drug from administered dosage form,
reaches the systemic circulation &
becomes available at the site of action

Bioavailable fraction (F), refers to the fraction of administered dose that enters the systemic circulation

$$F = \frac{\text{Bioavailable dose}}{\text{Administered dose}}$$

Therapeutic Relevance

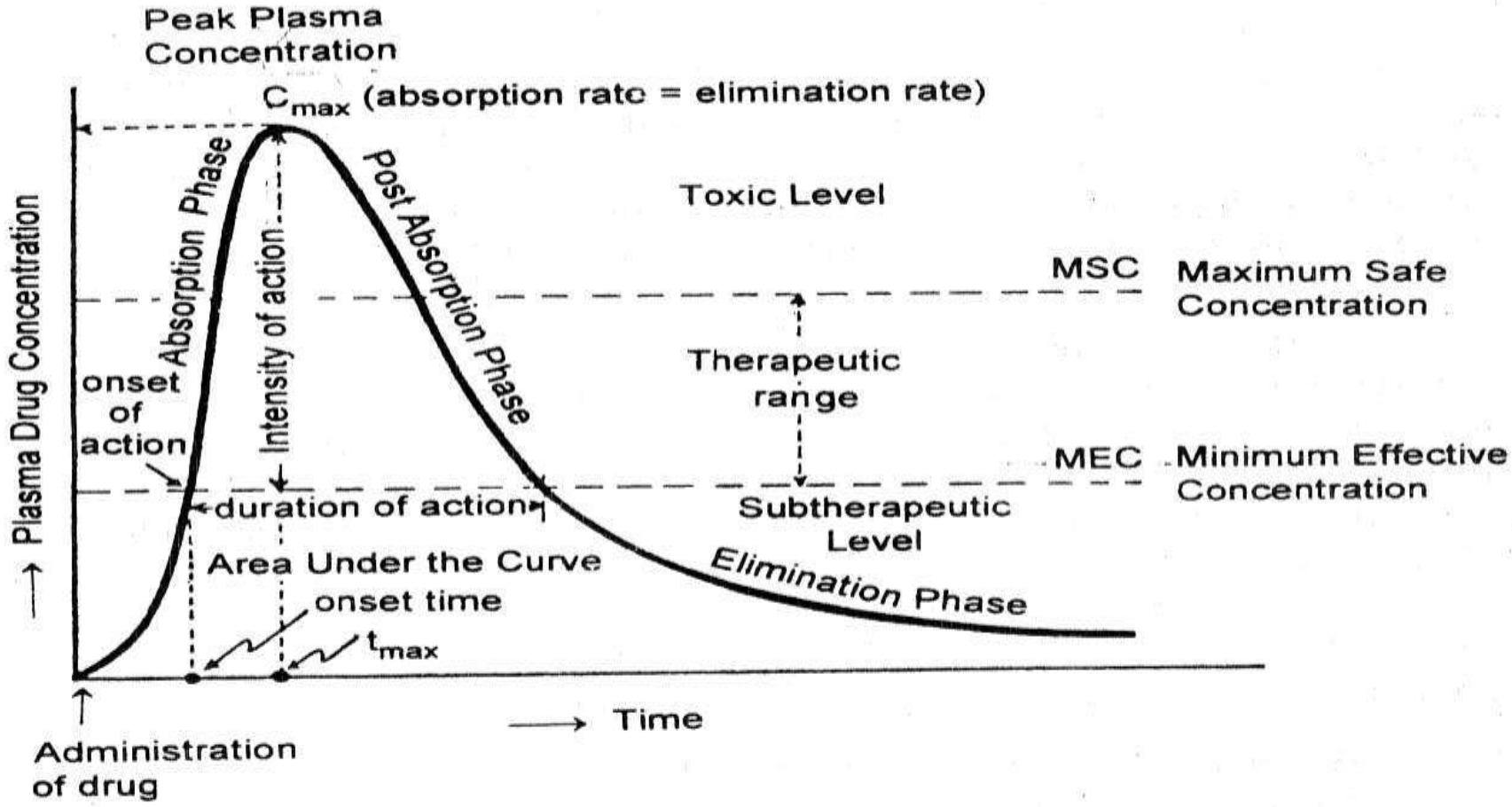


Fig. 9.1 A typical plasma concentration-time profile showing pharmacokinetic and pharmacodynamic parameters, obtained after oral administration of single dose of a drug.

➢ Absolute Bioavailability

Compares the bioavailability of the active drug in systemic circulation following non-intravenous administration with the same drug following intravenous administration

✓ For drugs administered intravenously, bioavailability is 100%

✓ Determination of the best administration route

$$F_{ab} = \frac{(AUC)_{drug}}{(AUC)_{IV}}$$

➢ Relative Bioavailability

Compares the bioavailability of a *formulation (A)* of a certain drug when compared with another *formulation (B)* of the same drug, usually an established standard

$$F_{rel} = \frac{(AUC)_{drug}}{(AUC)_{standard}}$$

Factors affecting Bioavailability of a Drug

➢ Physical properties of a drug

✓ **Physical state**:

• Liquids > Solids
[Solution > Suspension > Capsule > Tablet > Coated tablet]

• Crystalloids > Colloids

✓ **Lipid or water solubility**:

• Aqueous phase at absorption site

• Passage across Cell surface

➢ Dosage forms

✓ **Particle size**:

- Important for sparingly soluble drugs
- ↓ the size, ↑ the absorption, ↓ the dose
- Nano-crystalline formulations of Saquinavir
- If ↓ absorption needed (local action on GIT), ↑ the size

➢ Physiological factors

✓ **Ionization**:

• Unionized form penetrates the GI mucosal lining quickly

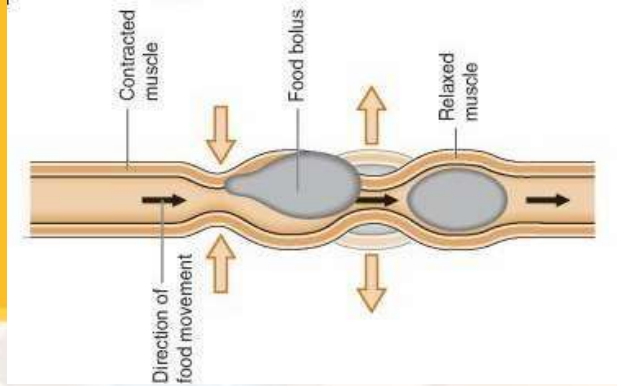
✓ **pH of the fluid**:

• Weakly acidic drugs: Aspirin, Barbiturates→ Stomach, duodenum

• Weakly basic drugs: Pethidine, Ephedrine→ Small intestine

• Strongly acidic / basic drugs: highly ionized & poorly absorbed

➢GI transit time



✓**Prolonged gastric emptying**:

• Delays absorption due to stasis
(e.g. with anticholinergics / Diabetic neuropathy)

✓**Increased peristaltic activity**:
(e.g. Metoclopramide→ speeds up the absorption of analgesics)

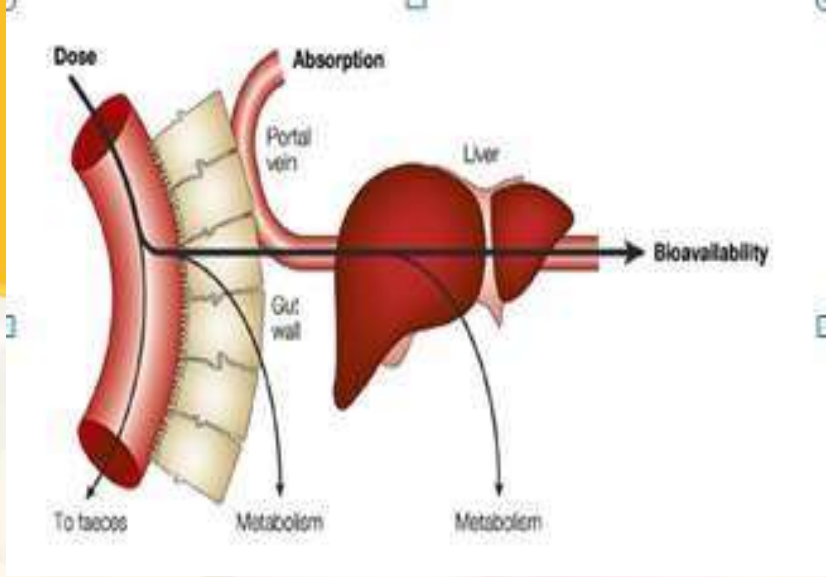
✓**Excessive peristaltic activity** (as in Diarrhoea) impairs absorption

✓**Fed state**:

• impairs progress of drug to intestine→ ↓ absorption (Indinavir)
• ↑ splanchnic blood flow→ ↑ absorption (Propranolol)

✓**First pass metabolism**:

- Gut wall (e.g. Isoprenaline)
- Liver (e.g. Opoids, ß-blockers, Nitrates)



✓**Presence of other agents**:

- Vitamin C ↑ Iron absorption, Phytates retard it
- Calcium ↓ absorption of Tetracyclines

✓**Disease states**:

- Malabsorption, Achlorhydria, Cirrhosis, Biliary obstruction can hamper absorption

✓**Entero-hepatic cycling**:

- Increases bioavailability (e.g. Morphine, OC pills)

Concept of Equivalents

➢ Pharmaceutical equivalents

✓ equal amounts of the identical active drug ingredient,
(i.e. the same salt or ester of the therapeutic moiety)

✓ identical dosage forms

✓ not necessarily containing the same inactive ingredients

➢ Pharmaceutical alternatives

✓ identical therapeutic moiety, or its precursor

✓ not necessarily the same:

- salt or ester of the therapeutic moiety
- amount
- dosage form

➢ Bioequivalence

- ✓ Pharmaceutical equivalent / alternative of the test product,
- ✓ when administered at the same molar dose,
- ✓ has the rate and extent of absorption
- ✓ not statistically significantly different from that of the reference product

➢ Therapeutic equivalence

- ✓ Same active substance or therapeutic moiety
- ✓ Clinically show the same efficacy & safety profile

- Strength of dosage form
- Excipients
- Other pharmaceutical factors

- Amount of drug released from the dosage form

Amount of drug absorbed from the dosage form

Concentration of drug in the central compartment

- Amount of drug in the body

- Concentration of drug at site of action

RESPONSE

- Patient related factors
- Administration related factors

- Strength of dosage form
- Excipients
- Other pharmaceutical factors

In vitro Quality Control testing

Amount of drug absorbed from the dosage form

In vivo Bioequivalence studies

- Amount of drug in the body

- Concentration of drug at site of action

PD studies/ Clinical Trials

- Patient related factors
- Administration related factors

Reference Product

- ✓ Identified by the Regulatory Authorities as "Designated Reference Product"
- ✓ Usually the Global Innovator's Product
- ✓ Protected by a patent
- ✓ Marketed under manufacturers brand name
- ✓ Clinical efficacy & safety profile is well documented in extensive trials
- ✓ All generics must be Bioequivalent to it
- ✓ In India, CDSCO may approve another product as Reference product

Generic Drug

✓ Drug product which is identical or bioequivalent to Brand/ Reference drug in:

- Active ingredient (s)
- Route of administration
- Dosage form
- Strength
- Indications
- Safety

✓ <u>May have different</u>:

- Inactive ingredients
- Colour
- Shape

✓ Almost half of drugs in market have Generics

Objectives of BA & BE Studies

- ✓ Development of suitable dosage form for a New Drug Entity
- ✓ Determination of influence of excipients, patient related factors & possible interactions with other drugs
- ✓ Development of new drug formulations of existing drugs
- ✓ Control of quality of drug products, influence of → processing factors, storage & stability
- ✓ Comparison of availability of a drug substance from different form or same dosage form produced by different manufacturers

When is Bioequivalence not necessary (Biowaivers)

a) Parental Solution; same active substance with same concentration, same excipient

b) Oral Solution; same active substance with same concentration, excipient not affecting GI transit or absorption

c) Gas

d) Powder for reconstitution as solution; meets criterion (a) or (b)

e) Otic/Ophthalmic/Topical Solution; same active substance with same concentration, same excipient

f) Inhalational Product/ Nasal Spray; administered with or w/o same device as reference product ; prepared as aqueous solution ; same active substance with same concentration, same excipient

NDA vs ANDA Review Process

NDA Requirements

1. Chemistry
2. Manufacturing
3. Controls
4. Labeling
5. Testing
6. Animal Studies
7. Clinical Studies
8. Bioavailability

ANDA Requirements

1. Chemistry
2. Manufacturing
3. Controls
4. Labeling
5. Testing
6. Bioequivalence

Orange Book

- ✓All FDA approved drugs listed (NDA's, ANDA's & OTC's)
- ✓ Expiration of patent dates
- ✓ <u>Drug, Price and Competition Act (1984)</u>
  FDA required to publish Approved Drug Products with Therapeutic Equivalence & Evaluations

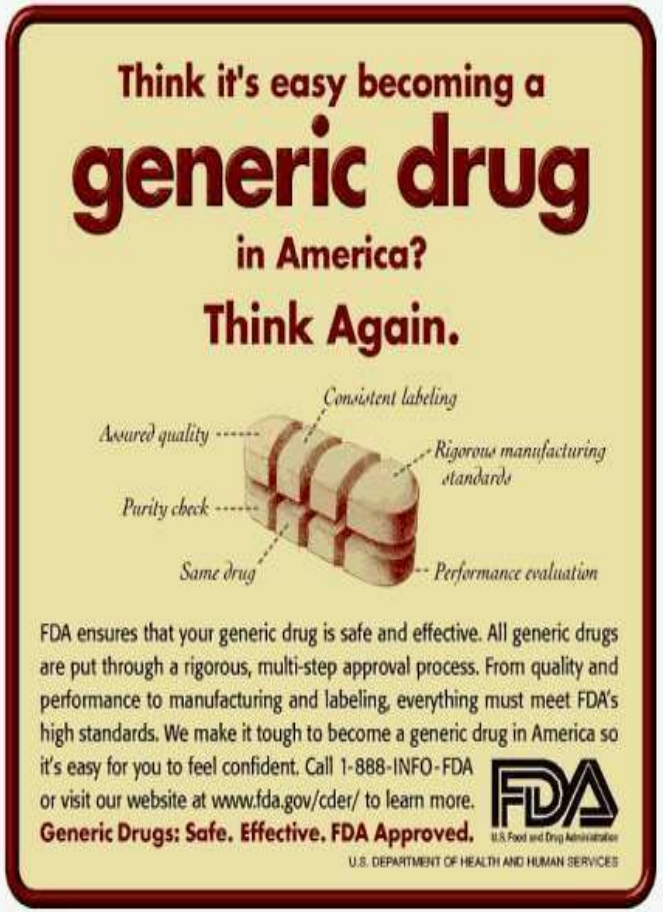
Methods used to assess Equivalence

I. Pharmacokinetic Studies

II. Pharmacodynamic Studies

III. Comparative Clinical Studies

IV. Dissolution Studies



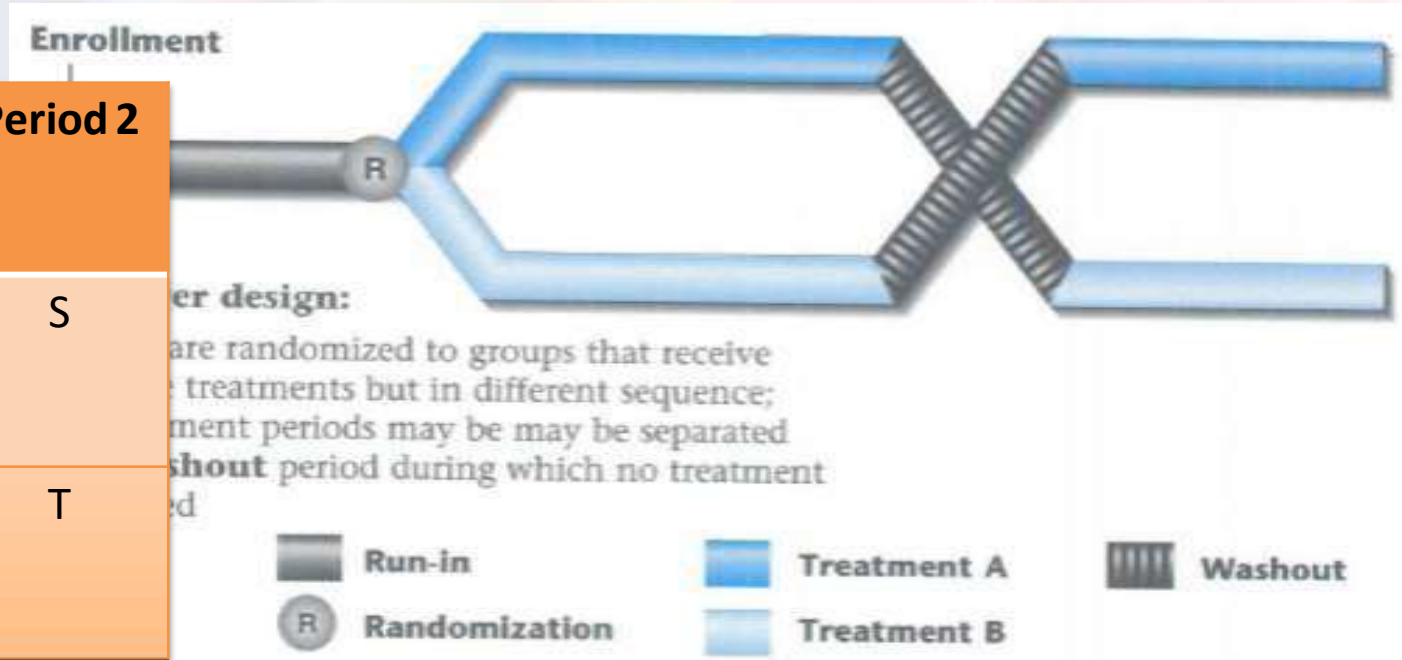
Pharmacokinetic Studies

I. Two-Period Crossover Design

✓2 formulations, even number of subjects, randomly divided into 2 equal groups

✓First period , each member of one group receive a single dose of the test formulation; each member of the other group receive the standard formulation

Subjects	Period 1	Period 2
1-8	T	S
9-16	S	T



II. Latin Square Design

- ✓ More than two formulations
- ✓ A group of volunteers will receive formulations in the sequence shown

Vol.No.	Period 1	Period 2	Period 3
1	A	B	C
2	B	C	A
3	C	A	B

III. Balance Incomplete Block Design (BIBD)

- ✓ More than 3 formulations, Latin square design will not be ethically advisable
- ✓ Because each volunteer may require drawing of too many blood samples
- ✓ If each volunteer expected to receive at least two formulation, then such a study can be carried out using BIBD

Vol. No.	Period 1	Period 2
1	A	B
2	A	C
3	A	D
4	B	C
5	B	D
6	C	D
7	B	A
8	C	A
9	D	A
10	C	B
11	D	B
12	D	C

IV. Parallel-Group Design

- ✓Even number of subjects in two groups
- ✓Each receive a different formulation
- ✓No washout necessary
- ✓For drugs with long half life

Treatment A	Treatment B
1	2
3	4
5	6
7	8
9	10
11	12

Parallel	Crossover
• Groups assigned different treatments	• Each patient receives both treatments
• Shorter duration	• Longer duration
• Larger sample size	• Smaller sample size
• No carryover effect	• Carryover effect
• Doesn't require stable disease & similar baseline	• Requires stable disease & similar baseline

V. Replicate Crossover-study design

✓For highly variable drugs

✓ Allows comparisons of within-subject variances

✓ Reduce the number of subjects needed

➢Four-period, two-sequence, two-formulation design (recommended)

OR

➢Three-sequence, three-period, single-dose, partially replicated

Period	1	2	3	4
Group 1	T	R	T	R
Group 2	R	T	R	T

VI. Pilot Study

✓ If the sponsor chooses, in a small number of subjects

✓ To assess variability, optimize sample collection time intervals & provide other information

✓ Example:

- Immediate-release products: careful timing of initial samples→ avoid a subsequent finding that the first sample collection, occured after the plasma concentration peak

- Modified-release products: determine the sampling schedule → assess *lag time* & *dose dumping*

✓ Can be appropriate, provided its design & execution are suitable & sufficient number of subjects have completed the study

Subject selection

- ✓Healthy adult volunteers
- ✓Age: 18-45 yrs
- ✓Age/Sex representation corresponding to therapeutic & safety profile
- ✓Weight within normal limits→ BMI
- ✓Women: Pregnancy test prior to 1st & last dose of study; OC pills C/I
- ✓Drug use intended in Elders (Age >60yrs)
- ✓Teratogenic Drugs→ Male volunteers
- ✓Highly toxic drugs: Patients with concerned disease (stable) eg. Cancer

Exclusion Criteria

- ✓H/o allergy to test drug
- ✓H/o liver or kidney dysfunction
- ✓H/o jaundice in past 6 months
- ✓Chronic diseases eg. Asthma, arthritis
- ✓Psychiatric illness
- ✓Chronic smoker, alcohol addiction, drug abuse
- ✓Intake of enzyme modifying drug in past 3 months
- ✓Intake of OTC/Prescription drugs past 2 weeks
- ✓HIV positive
- ✓BA & BE studies in past 3 months
- ✓H/o bleeding disorder

Selection of Number of Subjects

✓ Sample size is estimated by:

- Pilot experiment
- Previous studies
- Published data

✓ Significance level desired, usually 0.05

✓ Power of the study, normally 80% or more

✓ Expected deviation (Δ) from the reference product, as compatible with BE

✓ If no data available, reference ratio of 0.95 (Δ = 5%) used

- ✓ Minimum 16 subjects, unless ethical justification
- ✓ Allow for drop-outs
- ✓ Replace drop-outs→ substitute follow same protocol; similar environment
- ✓ Sequential/ Add-on Studies→ large no. of subjects required, results of study do not convey adequate significance

Genetic Phenotyping

✓Drug is know to be subject to genetic polymorphism

✓Cross-over design→ Safety & Pharmacokinetic reasons

✓All Parallel group design

✓Indian population:

- Captures genetic diversity of the world
- Forms continuum of genetic spectrum
- >1000 medically relevant genes

✓ Diverse patient/ volunteer pool for conducting BA & BE studies

Characteristics to be measured

✓Accessible biological fluids like blood, plasma &/or serum to indicate release of the drug substance from the drug product into the systemic circulation

✓Mostly: Active drug substance

✓Active / Inactive metabolite maybe measured in cases of:

- Concentration of drug too low
- Limitation of analytical method
- Unstable drug
- Drug with very short half life
- Pro-drugs

✓ Excretion of drug & its metabolites in urine→ Non-linear kinetics

✓ Measure individual enantiomers when they exhibit:

- Different pharmacokinetic/ pharmacodynamic properties
- Non-linear absorption
- Safety/Efficacy purposes

✓ Drugs that are not absorbed systemically from site of application *surrogate marker* needed for BA & BE determination

Parameters to be measured

✓Pharmacokinetic Parameters measured are:

- C_{max}
- T_{max}
- AUC_{0-t}
- $AUC_{0-\infty}$

$$AUC_{0-\infty} = AUC_{0-t} + C_{last}/k$$

For steady state studies:

- AUC_{0-t}
- C_{max}
- C_{min}
- Degree of fluctuation

Fasting & Fed State Conditions

➢ Fasting Conditions:

✓ Single dose study:

- Overnight fast (10 hrs) and subsequent fast of 4 hrs

✓ Multiple dose study:

- Two hours fasting before and after the dose

Thanks