Chapter

1

Introduction to Experimental Pharmacology

Specific Learning Objectives

- Definitions of common terminologies such as pharmacokinetics, pharmacodynamics, *ex vivo*, *in vitro*, *in situ*, *in vivo*, *in silico*.
- Contributions of pioneer scientists in experimental pharmacology.
- Brief understanding of pre-clinical and clinical pharmacology.

PHARMACOLOGY

- Branch of science which deals with the study of drugs on living system.
- The term pharmacology is derived from two Greek words—*Pharmacon* meaning 'a drug or medicine' and *logos* meaning 'a discourse or rational discussion'.
- Pharmacology is the scientific study of mechanisms by which drugs alter the biological system in an attempt to improve health and alleviate disease.

DRUG

- The term is derived from the French word *Drogue* meaning 'a dry herb'.
- Drug is a substance that is used for diagnosis, prevention or treatment of a disease.
- WHO (1966) has defined drug as "a substance or product that is used or intended to be used to modify or explore the physiological systems or pathological states for the benefit of the recipient".

- Two important aspects of pharmacology under which the properties and characteristics of a drug are described are: Pharmacokinetics and pharmacodynamics.

PHARMACOKINETICS

- The term is derived from the Greek word *kinesis* meaning 'movement'.
- It is the study of processes involved in movement of drug in, through and out of the body.
- It includes drug absorption, drug distribution, drug biotransformation or metabolism and drug excretion (ADME).
- In simple words, pharmacokinetics is what the body does to the drug.

PHARMACODYNAMICS

- The term is derived from the Greek word *dynamics* meaning 'power'.
- It is the study of physiological and biochemical effects of drugs including its mechanism of action and adverse effects.
- In simple words, pharmacodynamics is what the drug does to the body.
- It includes both the qualitative and quantitative aspects of drug action in the form of pharmacological effects and dose–response relationship, respectively.

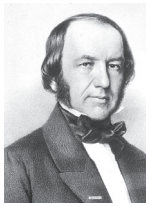
EXPERIMENTAL PHARMACOLOGY

- Study of the effects of various pharmacological agents on different animal species.
- Originating in the 19th century the discipline makes drug development possible.
- In the early 19th century, **Francois Magendie (1783–1855)** studied the action of nux vomica (a strychnine containing plant) on dogs and showed that spinal cord was its site of convulsant action. He is remembered as pioneer of experimental physiology. He described the median or

Francois Magendie
(1783–1855)

medial aperture or foramen of Magendie, an opening of the fourth ventricle at the caudal portion of the roof of fourth ventricle. Lesion of cerebellum can cause downward and inward rotation of eye, known as Magendie sign. He is also remembered for Bell-Magendie Law in neuroanatomy and neurophysiology that describes that anterior spinal nerve roots contain only motor fibres while the posterior roots only sensory fibres.

- **Claude Bernard (1813–1878)** discovered that the arrow poison curare acts at neuromuscular junction to block the neuromuscular transmission. He is remembered as father of physiology. His contributions include importance of pancreatic juice in digestion, glycogenic functions of liver and discovery of vasomotor system. He established the existence of both vasodilator and vasoconstrictor nerves.

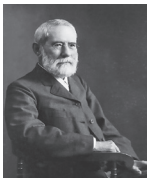


Claude Bernard
(1813–1878)

- **Rudolph Buchheim (1820–1879)** is remembered for his pioneer work in experimental pharmacology. His contributions to pharmacology include classification of drugs according to mode of action, pharmacological actions and through scientific experiments. He is also remembered for founding pharmacological laboratories and training to budding pharmacologists. He systematically explored experimental pharmacology.

Rudolph Buchheim
(1820–1879)

- **Oswald Schmiedeberg (1838–1921)** is remembered as Father of modern pharmacology. Initially, he worked as assistant to Rudolph Buchheim. He did extensive work on chemicals, poisons and hypnotics. He published over 200 articles and scientific books.



Oswald
Schmiedeberg
(1838–1921)

- **John Jacob Abel (1857–1938)** is considered as Father of American pharmacology. He established the

Pharmacology Department at John Hopkins University School of Medicine in 1893. He is remembered for the isolation of monobenzoyl derivative of epinephrine from adrenal medulla. Along with R G Rowntree and B B Turner, Abel devised a 'vividiffusion' apparatus which is presumed to be the precursor of modern day dialysis machine. His other contributions include crystallization of insulin and founding scientific journals related to biological chemistry, pharmacology and experimental therapeutics.

John Jacob Abel
(1857–1938)

- **Louis Lasagna (1923–2003)** is considered as Father of clinical pharmacology. He is the author of the book "The Doctors' Dilemmas" (1963). In 1964, Lasagna wrote a modern version of Hippocratic Oath. He conceptualized the placebo effect and controlled clinical trials. He was involved in US Federal Drug Regulation. Lasagna was interested in work on analgesia, euthanasia, hypnosis and the problems related to the research and development of new medicinal products. He determined the need for ethical and regulatory requirements in clinical research.

Louis Lasagna
(1923–2003)

- **Paul Ehrlich (1854–1915)** the Noble Prize-winning German Physician and Pharmacologist conceptualized the theory of magic bullet to treat infections. He is considered as the Father of chemotherapy and Father of immunology.

Paul Ehrlich
(1854–1915)

- **Colonel Sir Ram Nath Chopra (1882–1973)** is considered as Father of Indian pharmacology. He worked as Professor of Pharmacology at the Calcutta School of Tropical Medicine. He did pioneering work on herbal remedies including *Rauwolfia serpentina*.

Colonel Sir Ram
Nath Chopra
(1882–1973)

AIMS OF EXPERIMENTAL PHARMACOLOGY

1. To find out a therapeutic agent suitable for human use in preclinical studies.
2. To study the toxicity of drugs.
3. To study the mechanism of action and site of action of drugs.

TOXICOLOGY

- Toxicology is the scientific study of mechanisms by which drugs and chemicals in the environment produce unwanted effects.
- Toxicology as a scientific discipline overlaps biology, chemistry, medicine and pharmacology.
- It involves the study of adverse effects of chemical substances and drugs on living organisms and the practice of diagnosing and treating the same including the poisoning or overdose.
- Toxicology studies are carried out on all drug substances to ensure safety.

MEDIAN LETHAL DOSE (LD_{50})

It is the amount (dose) of a chemical that is lethal to one half (50%) of the experimental animals (usually mice or rats) exposed to it. LD_{50} values are usually expressed as the weight of the chemical substance per unit of body weight (mg/100 g or µg/kg or mg/kg or g/kg). LD_{50} values provide a measure of the acute toxicity of a substance. The lower the LD_{50} value, the greater is its toxicity.

COMMON TERMINOLOGY

1. ***Ex vivo*** (Latin—out of the living): Phenomenon that takes place outside the organism. *Ex vivo* refers to experimentation or measurements done in or on tissue from an organism in an external environment with minimal alteration of natural conditions. Examples: Bioassay, CAM (chick chorioallantoic membrane) assay, cell line culture, study of body fluid samples such as blood, plasma, CSF, urine, feces, bronchoalveolar lavage fluid and tissue extracts.

2. ***In vitro*** (Latin—within the glass): In simple terms "test tube experiments". These studies are conducted using components of an organism that have been isolated from their usual biological surroundings in order to permit a more detailed and convenient analysis. Examples: Cell culture, tissue culture, studies on sub-cellular components (e.g. ribosomes, mitochondria), studies on the purified molecules in test tubes (e.g. proteins, DNA, RNA) and IVF (*in vitro* fertilization). The main difference between *ex vivo* and *in vitro* assays is that in the *ex vivo* method, the tissue is directly taken from the living organism while in the *in vitro* method, it is simply a cell-system established in a cell culture laboratory.
3. ***In situ*** (Latin—on site or in place or in position): The phenomenon occurs at the same place without isolating it from other systems or altering the original conditions of the procedure (e.g. experiments on dissected or anaesthetized animals). In pharmacology, usually perfusion method studies are classified as *in situ* studies (e.g. effects of cardiac stimulants and depressants on the perfused frog heart, in which frog is dissected but the heart is not isolated). In oncology, *in situ* means that the malignant cells are present as tumour but have not metastasized. Example: Carcinoma-*in situ* (not having invaded beyond the basement membrane). This type of tumour can be removed by surgery.
4. ***In vivo*** (Latin—within the living): Phenomenon that is performed in a whole living organism (animal or human).

   Examples

   - Assessment of analgesic activity using tail flick analgesiometer or Eddy's hot plate method (rats or mice).
   - Assessment of locomotor activity using actophotometer (rats or mice).
   - Assessment of miotic, mydriatic and surface anaesthetic activity (rabbits).
   - Assessment of antianxiety activity using elevated plus maze apparatus (rats or mice).
   - Assessment of antipsychotic activity using Cook's pole climbing apparatus (rats or mice).

- Assessment of motor function and muscle strength using rota-rod apparatus (rats or mice).
- Assessment of antiepileptic activity using electroconvulsiometer (rats or mice).
- Human clinical trials.
- In microbiology, *in vivo* is often used to refer experimentation done in a whole organism, rather than in live isolated cells.

5. ***In silico*** (Latin—*in silicon*—derived from silicon in computer chips): The procedure is performed on computer or via computer simulation. *In silico* procedures are basically applied only to computer simulation of laboratory or natural processes and did not refer to calculations done by computer. *In silico* methods have high potential to speed the rate of drug discovery while reducing the need for expensive laboratory work. In many situations, *in silico* models can be used as alternative to *in vivo* methods. Computers can simulate human cells to examine the effect of particular drugs, toxicity and safety. The chief advantage is the rapid speed of the process at which it can be carried out and without inhumane procedures on the animals. Computers can store a large amount of data on previous drug development. This is especially valuable for new drug development and cost-effective. In the Covid-19 vaccines and drug development, *in silico* methods helped a lot. *In silico* methods are now a vital part of new drug development and success. Computers are able to predict the effect of new drugs without any harmful event on humans or animals. *In silico* methods predict toxicity through computing models, quantitative structure–activity relationships (QSARs), and algorithms with toxicity data. They use existing data derived from molecular structures to predict the toxicity and biological activities of a drug.

The conventional teaching methods in experimental pharmacology involved the use of experimental animals and procedures. Now the animal experiments for teaching purpose have shifted to simulation experiments on computer-assisted learning (CAL).

PRECLINICAL PHARMACOLOGY

- Preclinical pharmacology is a stage in drug development consisting of many activities that determine whether a new chemical entity (NCE) or drug is accepted for human clinical trials. In the drug discovery and development process, the preclinical pharmacology and toxicology play a critical role.
- It is also known as preclinical studies or nonclinical studies and conducted in laboratory animals. This is a stage of research that begins before the clinical trials (in humans). It involves pharmacokinetic, pharmacodynamic and toxicity studies. Both *in vitro* and *in vivo* studies are performed to know the mechanism(s) and site(s) of action of NCE.
- Preclinical animal studies are conducted in accordance to the standards of good laboratory practices (GLP). This ensures the reliability and reproducibility of laboratory data and minimizes human errors.
- The ultimate aim of preclinical studies is to determine the safety data from the toxicological and pharmacodynamic studies such as LD_{50}, ED_{50}, therapeutic index (TI), certain safety factor (CSF), and other safety parameters such as maximum tolerated dose (MTD), no adverse effect level dose (NoAEL) and human equivalent dose (HED). Pharmacokinetic data includes absorption, distribution, metabolism, excretion, relative bioavailability, elimination half life ($t_{1/2}$), etc.

CLINICAL PHARMACOLOGY

- It is the scientific study of drug in human (healthy volunteers or patients) for the evaluation of efficacy and safety. Systemic and scientific study of NCE in human after the successful and fruitful completion of preclinical studies is called clinical trials.
- The clinical trials in human involve four phases—I, II, III and IV, and should comply with the standards of Good Clinical Practice (GCP) prescribed by the International Council for Harmonization (ICH) and Declaration of Helsinki.

- The manufacturers file an investigational new drug (IND) application to the authorized Drug Regulatory Authority (DRA) of the respective country such as DCGI-CDSCO, New Delhi; India.
- **Phase I clinical trials** are non-blinded open trials on 25–100 healthy volunteers to determine safety, tolerability, PK and dosing.
- **Phase II clinical trials** could be early phase II which are done on up to 200 patients and are single blind while late phase II are double blind and conducted on 200–400 patients for the verification of safety and efficacy.
- **Phase III clinical trials** are large scale multicenter trials in 1000–5000 plus subjects involving heterogeneous population. Such studies are randomized, double-blind and cross-over to minimize human errors and bias.
- A **new drug application (NDA)** is submitted by the sponsor to the Drug Regulatory Authority of respective country, once the phase III clinical trials are completed satisfactorily. After the approval the new drug is launched.
- **Phase IV trials (post-marketing surveillance, PMS)** begins after the approval of new drug. Post-marketing studies involve the studies on pharmacoeconomics, pharmacoepidemiology, efficacy, safety, adverse effects and pharmacovigilance, drug interactions, additional uses and different formulations. Obviously phase IV studies comprise of largest sample size and population. Phase IV studies have no fixed duration.

QUESTIONS

Q1. Mention five pioneer scientists/researchers of experimental pharmacology and their contributions.

Q2. Comment on *in silico* studies in pharmacology and their significance.

Q3. Write short notes on:

i. *Ex vivo* studies

ii. *In vitro* studies

iii. *In situ* studies

iv. *In vivo* studies

Q4. Compare and contrast:

i. Preclinical and clinical pharmacology.

ii. *In vivo* and *in vitro* studies.