Unit-II

Preclinical screening of new substances for the pharmacological activity using in vivo, in vitro, and other possible animal alternative models.

- General principles of preclinical screening.
- CNS Pharmacology: behavioral and muscle coordination, CNS stimulants and depressants, anxiolytics, anti-psychotics, anti-epileptics and nootropics.
- Drugs for neurodegenerative diseases like Parkinsonism, Alzheimers and multiple sclerosis.
- Drugs acting on Autonomic Nervous System
GENERAL PRINCIPLES OF PRECLINICAL SCREENING
Topics to discuss

- Introduction
- Types of screening
- Characteristics of screening tests
- General principles of screening tests
- Primary screening - Observational parameters
- Drug discovery
Introduction

Screening

• In medicine, a strategy used in a population to identify an unrecognised disease in individuals without signs or symptoms

Preclinical screening

• A series of laboratory tests of a new drug on the animal subjects.
Introduction

- Designed to **distinguish useful from non-useful drugs** as rapidly, comprehensively and inexpensively as possible.
- Involves a **test or group of tests** which permits the detection of physiological activity.
- Determine whether the **new drugs are worth for further attention** & to indicate which among them have the **most interesting pharmacological property**.
- Range of **qualitative changes** produced by the drug & the **quantitative relation** between them.
# Types of screening

<table>
<thead>
<tr>
<th>Type of experiment</th>
<th>Meaning</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>In vivo</em></td>
<td>Within the living experimentation using a whole, living organism</td>
</tr>
<tr>
<td><em>In vitro</em></td>
<td>Within the glass i.e., in a test tube or petri dish</td>
</tr>
<tr>
<td><em>In situ</em></td>
<td>In position</td>
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<tr>
<td></td>
<td><em>in situ</em> means to examine the phenomenon exactly in place where it occurs (i.e. without moving it to some special medium)</td>
</tr>
<tr>
<td><em>In silico</em></td>
<td>performed on computer or via computer simulation</td>
</tr>
</tbody>
</table>
**Evaluation of Anti-epileptic activity:**

<table>
<thead>
<tr>
<th>In vivo</th>
<th>In vitro</th>
</tr>
</thead>
<tbody>
<tr>
<td>Electroshock in mice (grandmal epilepsy)</td>
<td>Electrical recordings from Hippocampal slices</td>
</tr>
<tr>
<td>Pentylene tetrazole (Metrazole) induced seizures in mice</td>
<td>Electrical recordings from isolated nerve cells</td>
</tr>
<tr>
<td>4-aminopyridine induced seizures in mice</td>
<td></td>
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<tr>
<td>Bicuculline Test in rats (GABA\textsubscript{A} antagonist)</td>
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</tr>
</tbody>
</table>
Schematic of decerebrate arterially perfused rat (DAPR) in situ preparation for bladder studies. Shows the preparation with a double lumen cannula.

In-silico studies
Types of screening

- Simple Screening
- Blind Screening
- Programmed screening
Types of screening

Simple Screening

- Screening is called simple when only one or two tests are used to find substances having a particular property.
- E.g. Test for sugar in the blood might be used to screen compounds for hypoglycaemic activity.
Types of screening

Blind screening

- New series of chemical substances obtained either through natural source or synthesis.
- Contain techniques for detecting pharmacological activity in a group of substances without pharmacological history.
- Requires considerable planning & skilful execution of the tests in order to be economical of time and money.
- No assumptions are made about what the probable actions of a compound may be (except when an already carefully studied series of compounds having similar structures have been investigated)
Types of screening

Programmed Screening

- When a new drug of a specific type or when a series of compounds are to be investigated for some pharmacological activity, a program of testing is identified, this could limit the screening procedure for particular tests that could provide greater precision.
- Provide indications for potential side effects.
- Screening tests are designed & performed to identify agents having a certain set of characteristics that will either exclude them from further consideration or cause them to be selected for closer attention.
Characteristics of screening tests

- Sensitivity
- Specificity
- Positive Accuracy
- Negative Accuracy
- Capacity (no. of compounds that can be evaluated)
- Reproducibility (probability that a screening test will produce the same result at another time)
**What is Sensitivity?**

\[
\text{Sensitivity} = \frac{\text{Number of True positive test}}{\text{(Number of True positive + Number of False negative)}}
\]

**What is Specificity?**

\[
\text{Specificity} = \frac{\text{Number of True Negative test}}{\text{Number of True negative + Number of False positive}}
\]

**What is False positive?**

**What is False negative?**

Positive Predictive Value (PPV) and Negative Predictive Value (NPV)

### Examples

<table>
<thead>
<tr>
<th>Positive Predictive Value (PPV)</th>
<th>Negative Predictive Value (NPV)</th>
</tr>
</thead>
</table>

### Calculations

**Actual Results**

**Positive**

- **True Positive**
  - The number of observations the model predicted were positive that were actually positive

- **False Negative**
  - The number of observations the model predicted were negative that were actually positive

**Negative**

- **False Positive**
  - The number of observations the model predicted were positive that were actually negative

- **True Negative**
  - The number of observations the model predicted were negative that were actually negative
General principles of screening tests

- Screening tests should always focus on detecting a single point of effect (such as mutagenicity, lethality, neurotoxicity).
- Evaluate large number of compounds with ease and speed of performance.
- Screening test must be very sensitive in its detection.
- Screening test should use small amounts of compound.
- Any screening system should be validated initially using a set of blind (positive & negative) controls. These blind controls should also be evaluated in the screening system to ensure continuing proper operation of the screen.
- Proper dose selection is essential for effective and efficient screen design and conduct.
General principles of screening tests

Tests on isolated organs bacterial cultures etc.
• These are preliminary tests to detect specific activity, e.g. antihistaminic, antisecretory, antibacterial etc.

Tests on animal models of human disease:
• These tests are conducted on animal models of human disease.
• The disease may be induced experimentally
• e.g. Alloxan induced diabetes in rats, Pentylenetetrazole induced convulsions.
General principles of screening tests

General Observational Test:

- Performed either in the beginning (in case of totally novel compounds) or after detecting useful activity in screening test, the drug is injected in tripling doses to small group of mice which are observed for overt effects.
- Preliminary ideas are drawn from the profile of effects observed. (FOB test/Irwin test)
General principles of screening tests

Confirmatory tests and analogues activities

- Compounds found active are taken up for detailed study by more elaborate tests which confirm and characterise the activity.
- Other related activities e.g. Antipyretic and anti-inflammatory activity in an analgesic are tested.

Mechanism of action

- Attempts are made to find out the mechanism of action.
- e.g. whether an antihypertensive is an $\alpha$-blocker/$\beta$-blocker/Calcium channel blocker/ACE Inhibitor/Centrally acting etc.
General principles of screening tests

Systemic Pharmacology

- Effects on major organ systems such as nervous, cardiovascular, respiratory, renal, GIT by the drug under development are worked out.

Quantitative tests

- Dose-response relationship
- Maximal effect & Comparative efficacy with other existing drugs are ascertained
General principles of screening tests

Pharmacokinetics

- Absorption
- Tissue distribution
- Metabolism
- Excretion
- Volume of distribution &
- Half-life of the drug are quantified
General principles of screening tests

Toxicological evaluation of new drugs

- Acute Toxicity studies
- Dose-ranging Studies
- Sub-acute Toxicity Studies
- Chronic Toxicity Studies
- Carcinogenicity Studies
- Reproduction Studies
- General Reproduction & Fertility
- Teratogenicity
- Perinatal & Postnatal Studies
- Genotoxicity Testing
- Metabolic Activation
- Special Studies
- Mutagenicity
- Immunological Toxicity
- Nephrotoxicity
- Behavioural Teratological Effect
Primary screening

• **Primary screening** of an unknown drug is the first step to check the effect of the drug on the central nervous system and autonomic nervous system.

• Observing the simple behavioral changes of the animals can assess activity of the drug.

• First step to evaluate the toxicity profile of any drug and help to categories the drug as safe or not and how the drug affect the nervous system, also in the development of new drug.

• Explore the onset of action and duration of action of a drug.

• Rodents mainly females are preferred choice of animal of primary screening.

• First four hours is important for the observation of behavioral changes of animals after administration of drug.
Primary screening - Observations

Awareness:
- Alertness
- Visual placing
- Passivity
- Stereotypy

Mood:
- Grooming
- Vocalization
- Restlessness
- Irritability

Motor activity:
- Reactivity
- Spontaneous activity
- Touch response
- Pain response

Motor incoordination:
- Staggering gait
- Abnormal gait
- Righting reflex

November 4, 2020
Preclinical Screening
Primary screening- Observations

Posture:
- Body posture
- Limb posture

Reflexes:
- Pinna reflex
- Corneal reflex

Muscle tone:
- Limb tone
- Grip strength
- Body tone
- Abdominal tone

CNS excitation:
- Straub’s tail
- Tremors
- Twitches
- Clonic/Tonic convulsions
Primary screening- Observations

ANS:
- Pupil size
- Palpebral opening
- Urination
- Salivation
- Piloerection
- Hypothermia
- Skin colour
- Heart rate
- Respiratory rate

Miscellaneous:
- Abnormal secretion
- Death
  (Acute/Delayed)
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<td>Twitching</td>
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<td>4</td>
<td>Rigidity</td>
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<td>6</td>
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<td>Sleep (Loss of R.R)</td>
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<td>Abnormal secretion</td>
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<tr>
<td>24</td>
<td>Death</td>
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</table>
**Hyper activity:** Excessive or abnormally increased muscular function or activity, similar to attention-deficit or hyperactivity disorder

**Pilo-erection:** Erection of hair or fur of the animal due to secretion of adrenalin (hyper secretion) & anxiety.

**Twitching:** A brief, contractile response of skeletal muscle elicited by a single maximal volley of impulses in the neurons supplying to it.

**Rigidity:** Inflexibility of stiffness. It may be due to increased acetyl choline secretion or activity as a result of lesions in the upper brain stem or neuron.

**Irritability:** The quality of being irritable, it’s a mood disorder. An abnormal behavior of animal can be observed.

**Jumping:** The skipping of several steps in a series, due to hyperactivity.

**Clonic convulsion:** An involuntary alternating series of contraction and relaxation of the voluntary muscle with or without loss of consciousness.

**Tonic convulsion:** Increased tonicity of the muscle with or without loss of consciousness, a type of convulsion comes in generalized convulsions.

**Ptosis:** Downward displacement of something, generally paralytic drooping of the upper eyelid.

**Sedation:** The allaying of irritability or excitement, but subject remains in conscious and rendered free from anxiety.

**Sleep (loss of R.R.):** It’s an unconscious state and subjected to be conscious by strong stimuli, with movement of eye ball (deep sleep)

**Loss of traction:** Loss of pulling or the act of drawing its own body part due to loss of muscle coordination.

**Loss of pinna-reflex:** Loss of touch sensitivity of the flap of the ear.

**Loss of pupil-reflex:** Loss of sensitivity for light reflex of the pupil.

**Catatonia:** A wide group of motor abnormalities, most involving extreme under or over activity, associated with primary catatonic schizophrenia.

**Ataxia:** Failure of muscle coordination, irregularly of muscle action, an unsteady and uncoordinated walk, employing a wide base and feet thrown out (loco motor activity).

**Loss of muscle tone:** Loss of muscle coordination can be described by loss of grip strength.

**Analgesia:** Loss of pain and reduced sensitivity to nociception or noxious stimuli, due to peripheral or central block of nerve impulse.

**Straub tail:** Due to anococcygeal muscle tonicity the tail of the animal will shape like letter “s” vertically, due to the opioid receptor activity.

**Laboried respiration:** Voluntary respiration or the forced respirations.

**Cyanosis:** A bluish discoloration of skin and muscle membranes due to excessive concentration of reduced hemoglobin in the blood.

**Blanching:** Expulsion of air by mouth, due to reflex mechanism, as a result of enteric, nervous system irritated by indigestion or hyper acid secretion in the stomach.

**Reddening:** Due to excessive peripheral blood flow and dilation of the peripheral blood capillary as a result of adrenalin over secretions.

**Abnormal secretions:** Increased or decreased secretion by the glands due to hypo or hyper activity of acetyl choline.
Drug discovery

• Starting point – several ways
• Genesis of research programme – five procedures
  ◦ Random screening
  ◦ Molecular manipulation
  ◦ Molecular designing
  ◦ Metabolites of drugs
  ◦ Serendipity
Random screening

- Battery of screening tests-different biological activities
- Animal behavior, isolated tissues, intact animals and animal models of disease.
- Blind hitting to hit a nail head
- Me too drugs- add to existing drug explosion
Molecular manipulation

- **Advantages** - better absorption, greater potency, more selective action and fewer side effects
- Thiazides and oral hypoglycemic sulphonyl ureas – modifications of old antibacterial drug sulphanilamide
- Synthetic penicillins and cephalosporins
Molecular designing

- Most rational form of drug research and development
- Designing of substances to fulfill biological task
- Synthesis of naturally occurring substance, a hormone or neurotransmitter
- Dopamine for cardiogenic shock, allopurinol for gout, levodopa for parkinsonism
Metabolites of drugs

- Active metabolites-therapeutic advantages over parent cpd.
  - *Paracetamol metabolite of phenacetin*- no renal damage
  - Oxazepam from chlordiazepoxide – shorter action

![Chemical structure of phenacetin and acetaminophen](image-url)
Serendipity

- Happy observation by chance
- New uses are found for old drugs and side effects find therapeutic applications

<table>
<thead>
<tr>
<th>Drug</th>
<th>Original use</th>
<th>Side effect</th>
<th>Derived use</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sulphanilamide</td>
<td>Antibacterial</td>
<td>Hypoglycemia</td>
<td>Sulphonyl ureas</td>
</tr>
<tr>
<td>Iproniazid</td>
<td>Antituberculosis</td>
<td>Euphoria</td>
<td>Antidepressants</td>
</tr>
<tr>
<td>Chlorpromazine</td>
<td>Antihistamine</td>
<td>Sedation</td>
<td>Antipsychotics</td>
</tr>
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