VARIOUS APPROACHES TO DRUG DISCOVERY

INTRODUCTION

- The various approaches to drug discovery include

1. Pharmacological
2. Toxicological
3. IND application
4. Drug characterization
5. Dosage form

- STEPS 1 and 2 constitute the PRECLINICAL STUDIES

PRECLINICAL TRIALS

- **Preclinical trial -** a laboratory test of a new drug or a new medical device, usually done on animal subjects, to see if the hoped-for treatment really works and if it is safe to test on humans.

PHARMACOLOGICAL APPPROACHES TO DRUG DISCOVERY

- Pharmacology as an academic principle can be loosely defined as the study of effects of chemical substances on living systems.
- This definition is so broad that it encompasses all the aspects of drug discovery, ranging from details of interaction between drug molecule and its target to consequences of placing the drug in the market

- Pharmacological research started in Europe in the second half of the 19th century when their founders investigated the action of existing drugs in animal experiments.

- Many new drugs were discovered by this classical approach during the 20th century.

- Measurement of dose response curves, effects over a given period of time and comparison of the effects after intravenous and oral administration already give hints for pharmacokinetic data.

- This approach has a disadvantage that it is time consuming and requires relatively large amount of the new compound.

- In the mid-1970's receptor binding assays were introduced.
- Many receptors have been identified by DNA sequencing technology, mostly belonging to the G-protein coupled receptor superfamily, for which ligands have not yet been identified.
- Reverse molecular pharmacology and functional genomic strategies are recommended to identify the activating ligands of these receptors.

- The ligand binding assay is a powerful tool in the search for agonists and antagonists for novel receptors, and for identification of novel classes of agonists and antagonists for known receptors.

- Specific and general pharmacological studies should be conducted to support use of therapeutics in humans

Components of pharmacological evaluation

1.Selectivity testing.

2. Pharmacological profiling.

3.Testing in animal models of disease.

4.Safety pharmacology.

SELECTIVITY TESTING

- The selectivity testing mainly involves 2 main stages:

1. **Screening for selectivity**
2. **Binding assays.**

Screening for selectivity

- The selectivity of a compound for a chosen molecular target needs to be assessed because it determines the potency of the drug.
- A selected compound may bind to molecular targets that are related or unrelated to the chosen molecular target thereby causing unwanted side effects.

Binding assays

- The aim of carrying out binding assays is to determine the dissociation constant of the test compound as a measure of affinity to the receptor.
- These assays are generally done with membrane preparations made from intact tissues or receptor expressing cell lines.
- In most cases the assay measures the ability of the test compound to inhibit the binding of a high affinity radioligand which selectively combines with the receptor in question.

PHARMACOLOGICAL PROFILING

- *Pharmacological profiling refers to determining the pharmacodynamic effects of a new compound. Either on:*

1. ***In vitro models:*** *Cell lines or isolated tissues.*
2. ***In vivo models****: Normal animals, animal models of disease*.*

The aim of pharmacological profiling is to answer the following questions:

- Does the molecular and cellular effects measured in screening assays actually give rise to the predicted pharmacological effects in intact tissues and whole animals?
- Does the compound produce effects in intact tissues or whole animals not associated with actions on its principle molecular target?

- Is there a close similarity between potency of the drug at molecular level, tissue level and the whole animal level.
- Do in vivo duration of action match up with the pharmacokinetic properties of the drug.
- What happens if the drug is continuously or repeatedly given to an animal over a course of days or weeks. Does it loose its effectiveness or reveal effects not seen on acute administration and whether there is any rebound after effect when it is stopped.

In vitro profiling

- In vitro profiling involves the studies on isolated tissues.
- This technique is extremely versatile and applicable to studies on smooth muscle* as well as cardiac and striated muscle, secretory epithelia, endocrine glands, brain slices, liver slices.
- In most cases tissue is obtained from a freshly killed or anaesthetized animal and suspended in warmed oxygenated physiological fluid solution.

Advantages

- The concentration-effect relationship can be accurately measured.
- The design of the experiments are highly flexible allowing measurement of:

- Onset and recovery of drug effects.
- Measurements of synergy and antagonism by other compounds.

Disadvantages

- The tissues normally have to be obtained from small laboratory animals, rather than humans or other primates.
- The preparations rarely survive for more than a day, so only short experiments are feasible.

In vivo profiling

- In vivo profiling involves the testing on normal animal models.
- These methods are time consuming and very expensive.
- They can be done on larger animals.
- A particularly important role of in vivo experiments is to evaluate the effects of long term drug administration on intact organism.

SPECIES DIFFERENCES

- It is important to take species differences into account at all stages of pharmacological profiling.
- The same target in different species will generally differ in its pharmacological specificity.
- The growing use of transgenic animal models will undoubtedly lead to an increase in animal experimentation

- Here there involves the use of animal models with the human disease for which the drug has been prepared.
- There tests are done to answer a crucial question to whether the physiological effects result in a therapeutic benefit.
- Despite the range of diversity of animal models from humans these tests will provide a valuable link to the chain of evidence.

TYPES OF ANIMAL MODEL

Animal models of disease can be broadly classified into

1. Acute physiological and pharmacological models
2. Chronic physiological and pharmacological models
3. Genetic models

Acute physiological and pharmacological model

- These models are intended to mimic certain aspects of the clinical disorder. The examples are:
- Seizures induced by electrical stimulation of brain as a model of epilepsy
- The hot plate for analgesic drugs as a model of pain.
- Histamine induced bronchoconstriction as a model of asthma.

Chronic physiological and pharmacological model

- These models involve the use of drugs or physical interventions to induce an ongoing abnormality similar to clinical condition. The examples are:
- The use of alloxan to inhibit insulin secretion as a model of TYPE I diabetes mellitus.
- Self administration of opiates, nicotine or other drugs as a model of drug dependence.

Genetic animals

- These are transgenic animals produced by deletion or over expression of specific genes to show abnormalities resembling the human disease.
- The development of transgenic technology has allowed inbred strains to be produced with the gene abnormality to be present throughout the animals life.
- More recent developments allow more control over timing and location of the transgenic effect.

VALIDITY CRITERIA IN CONTEXT TO ANIMAL TESTING

An animal model produced in a lab can never exactly replicate a spontaneous human disease state . so certain validity criteria have been set up, they are:

1. ***Face validity***
2. ***Construct validity***
3. ***Predictive validity***

1. FACE VALIDITY:

   This validity refers to the accuracy with which the model reproduces the phenomena(symptoms, clinical signs and pathological changes) characterizing the disease.

2. CONSTRUCT VALIDITY:

   This refers to the theoretical rational with which the model is based i.e. the extent to which the etiology of the human disease is reflected in the model.

3. PREDICTIVE VALIDITY:

- This validity refers to the extent to which the effect of manipulations(e.g. drug treatment) in the model is predictive of effects in the human disorder.
- This is the most important of the 3 as it is most directly relevant to the issue of predicting therapeutic efficacy.

SAFETY PHARMACOLOGY

- Safety pharmacology is the evaluation and study of potentially life threatening pharmacological effects of a potential drug which is unrelated to the desired therapeutic effect and therefore may present a hazard.
- These tests are conducted at doses not too much in excess of the intended clinical dose.

- Safety pharmacology seeks to identify unanticipated effects of new drugs on major organ function(i.e. secondary pharmacological effects).
- It is aimed at detecting possible undesirable or dangerous effects of exposure of the drug in therapeutic doses.
- The emphasis is on acute effects produced by single-dose administration rather than effects on chronic exposure as in toxicological studies.

AIM OF ESSENTIAL SAFETY PHARMACOLOGY

- To study the effects of the test drug on vital functions.
- Vital organ systems such as cardiovascular, respiratory and central nervous systems should be studied.
- Essential safety pharmacology studies may be excluded or supplemented based on scientific rationale

TYPE	PHYSIOLOGIAL SYSTEM	TESTS
CORE BATTERY	*CENTRAL NERVOUS SYSTEM*	Observations on conscious animals
		•Motor activity
		•Behavioral changes
		•Coordination
		•Reflex responses
		•Body temperatures
	CARDIVASCULAR SYSTEM	On anaesthetized animals
		•Blood pressure
		•Heart rate
		•ECG CHANGES
		Tests for delayed ventricular repolarisation
	RESPIRATORY SYSTEM	Anaesthetized and conscious
		•Respiratory rate
		•Tidal volume
		•Arterial oxygen saturation

TYPE	PHYSIOLOGIC SYSTEMS	TESTS
FOLLOW- UP TESTS	*CENTRAL NERVOUS SYSTEM*	•Tests on learning and speech
		•More complex tests for changes in behavior and motor function.
		•Tests for visibility and auditory function
	CARDIOVASCULAR SYSTEM	•cardiac output
		•Ventricular contractility
		•Vascular resistance
		•Regular blood flow
	RESPIRATORY SYSTEM	•Airway resistance and complince
		•Pulmonary arterial pressure
		•Blood gases

TYPE	PHYSIOLOGIC SYSTEM	TESTS
SUPPLEMENTARY TESTS	*RENAL FUNCTION*	•Urine volume, Osmolality, PH, •Proteinuria
		•Blood Urea/Creatinine
		•Fluid/Electrolyte balance
	AUTONOMIC NERVOUS SYSTEM	•C.V.S, Gastrointestinal and respiratory system responses to agonists and stimulation of autonomic nerves.
	GASTROINTESTINAL SYSTEM	•Gastric secretion
		•Gastric PH
		•Intestinal motility
		•Gastrointestinal transit time

- ***Toxicological approach to drug discovery***

- Drug discovery and development is a dynamic process, requiring integration of activities across a number of preclinical scientific disciplines for successful progression of therapeutic candidates.

- This review provides an overview of the early involvement of investigative toxicology in decision making during the drug discovery process.

- It is estimated that only between one in ten to one in one hundred chemicals identified as 'lead' compounds in early discovery actually makes it to the first step in clinical development (Phase I studies).

- Based on analysis of sample company data, 23% of clinically tested compounds achieve successful new drug application (NDA) approval.

- A strategy that incorporates toxicology early in the 'lead' compound selection process should help reduce risk and prove cost effective.

Regulatory toxicology

- To achieve successful approval to market a NCE as a drug, the drug must be shown to be clinically effective and potent; it must also be shown in repeated batches or lots of product to be pure, and it must be safe for the intended therapeutic indication.

- The obligation of establishing safety in the development process is to assure physicians, patients, drug regulatory agencies and the company that the compound can be used safely in patients for the intended duration necessary to achieve therapy.

- A series of regulatory toxicology requirements must be met in order to register a NCE for use in humans.

Investigative toxicology early in drug discovery

- The most significant challenge of discovery pharmacology and medicinal chemistry is the early identification of a lead drug candidate that might satisfy efficacy and safety hurdles to become a successful pharmaceutical agent.

- Examination of the specific chemical structure or class may be useful in predicting potential toxicity, and in the design of prescreens for candidate selection.

- Numerous analogues are 'screened' for activity using *in vitro* models that mimic a specific target in the disease process and which are predictive surrogates for biological activity.

- Successful 'hits' from *in vitro* screens are then evaluated to identify efficacy using *in vivo* models.

- It is at this point that discovery toxicology efforts can contribute significantly to identify a lead compound with the most desirable safety to efficacy profile, including absence of undesired toxicities, and best therapeutic index.

- Toxicologists in the pharmaceutical industry are responsible for identifying and characterizing each new drug candidate's toxicity *in vivo* in mammalian organisms and, where appropriate, *in vitro*, in cells/cellular constituents in three categories:

1) as side effects unrelated to the chemical's direct pharmacological action
2) as a result of exaggerated exposure or
3) as toxic effects which may be further characterized by appropriate testing

- A compound may cause deleterious effects that are target-organ specific, as for example affecting only the liver or the kidney.
- This may be attributed specifically to the chemical structure of the parent NCE or a toxic metabolite.
- Toxic effects occur as a result of both dose and exposure, with the mechanism of toxicity being often poorly understood.

- Among the first studies performed are genetic toxicology and acute tolerance testing.

Genetic toxicology testing system

- The test systems are among those more commonly used tests in the pharmaceutical industry to assess potential risk of genetic damage in man.

- They include one bacterial mutagenicity test, two additional *in vitro* tests using a mammalian cell system to assess chromosomal aberrations and mutations, and an *in vivo* dosing test to assess chromosomal aberrations in mouse bone marrow erythroid cells.

- Other test systems provide additional specific information and are used for research purposes.

- The purpose of assessing compounds in multiple tests is to detect the diverse kinds of mutations or chromosomal damage that may impact on human exposure by exposing sensitive cell systems and whole organisms to the compound.

- In practice, *in vitro* tests conducted ahead of *in vivo* studies to ensure that a chemical mutagen is identified as soon as possible, before extensive animal testing is begun or industrial worker exposure can occur.

Acute tolerance testing

- After a review of company and published literature for a particular chemical NCE the initial testing is done in rodents, usually rats, and is referred to as the acute tolerance test.

- This type of testing is no longer done in large animals.

- The LD50 has been the standard for defining acute toxicity for many years, but has been recently replaced by a number of procedures that approximate lethality using a fraction of the animals.
- A limit test of 2 g/kg of test article is used.
- If the test compound is non-toxic 14 days after dosing three female rats at 2 g/kg, this limit dose is repeated once again in three additional female and six male rats.
- Tolerance testing stops here if the compound shows no toxicity .

- If any form of toxicity is observed then the compound is placed in a dose range study.
- The dose range study is initiated for compounds where pharmacology data or literature references provide the toxicologist with a starting point on toxic dosage estimation.
- The goal of the those range tolerance study is to identify the minimal lethal dose (MLD), the maximum tolerated dose (MTD), and a no-effect dose with an approximate lethality slope.
- The final step in the tolerance study is to corroborate the absence of an obvious sex difference in the rat by repeating, in male animals, the immediate sublethal dose as determined in females.

- In summary, the acute tolerance study provides important in-life toxicity information early in pharmaceutical discovery testing.

- With these results the toxicologist:

1) determines the dosing range (< =MTD) for the rodent repeat dose study protocol (2 weeks)

2) has a starting point for non-rodent species dose range studies and

3) provides relevant information for accidental exposure of laboratory and manufacturing workers.

- Advances in computer technology have revolutionized the way toxicology departments collect, manage, and analyze the volumes of data generated.

- Information technology can be an effective 'weapon' to assist in maximizing development speed.

- The impact of this technology has dramatically reduced the time required to collect, verify, analyze and report data while improving data integrity by minimizing the amount of manual data manipulation.

- The ultimate goal is to achieve electronic submission of all toxicology study data to regulatory agencies to shorten the review time and improve the quality of the application.

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Thank you