Drug Characterization

- Drug characterization involves finding of biological and physiochemical properties of potential drug molecule.

- Two types of DC:

1. Biological characterization
   Interaction of drug molecule with biological systems

2. Chemical characterization:
   Physical and chemical characterization of a potential drug

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#Biological characterization:

Biological characterization is needed to assess whether the molecule will likely work in humans.

- Goals of biological characterization:
  - a-To find efficacy
  - b-Assess toxic effects

- Studied at the level of biochemical, cell, tissue, organ and organism level.

- Biochemical assays:
  - 1. Mechanism of action at a molecule level
    - Receptor binding (affinity and selectivity) assays
    - Enzyme inhibition. activity: Km, selectivity
  - 2. Metabolic processing - metabolic activation

- Cellular assays:
  1. Cell cultures: Cell penetration, receptor activity, transport
  2. Isolated tissues: Effects on specific tissue - possible toxicity
  3. Liver tissues (microsomes): Drug metabolism, drug interaction

Systemic / whole animals:

→ Rat, mouse: toxicity, LD_{50}, organ-specific efficacy

→ Dog, rabbit, guinea pig, monkey: pharmacokinetic, BA, toxicity

→ Lab animal models for disease: efficacy, toxicity

#Chemical Characterization:

Physical and chemical properties of a drug are critical for knowing how it can be formulated/stored/ administrated.

→ Chemical structure: Mass spectroscopy, NMR, X-ray crystallography

→ Solubility, pK_a

→ Partition coefficient: octanol: water

→ Stability

→ Impurities: TLC, HPLC, GC, mass spectroscopy

→ ADME

• Physico chemical properties are closely related to drug absorption and their values are used in predicting bioavailability and interpreting other in vitro and in vivo findings. Different approaches have been developed to address these properties at different stages in drug discovery, including hit finding, lead finding and optimization and nomination for development condition.

Main sources of drug leads :-

- 1970's and 1980.
  - Around 1970 large empirically based screening programs
  - From then on knowledge base grew for rational drug design.
  - Most leads had already been in a range of physical properties previously known to be consistent with oral activity.
- 1989 and on
  - High through screening of hundreds of thousands of compounds across in vitro assay.
  - Soon after combination chemistry.
  - Rapid progress in molecular genetics expression of receptors.
  - Drugs were dissolved in DMSO.(dimethyl sulfoxide)
  - The recent trend in medical chemistry leads towards larger molecule and increased Lipophilicity.
  - The candidate with the right balance of activity and availability is more desirable.

One further aspects is that "drug-likeness" profile of oral drugs is far beyond these simple rules and includes properties such as dissolution, absorption, metabolism, clearance and protein binding. A good drug candidate needs to have favorable above mentioned properties to ensure good pharmacokinetic (PK) outcome.

ADME and PK properties

Property	Describes	Comment	Models
Passive transmembrane diffusion	Ability to cross a membrane bilayer in a concentration gradient-driven fashion	Usually the major component of cell permeability and drug absorption	IAM, PAMPA
Cell permeability	Ability to traverse an Intact cell monolayer unchanged in an in vitro system	For compounds stable to first pass effect and with no solubility and dissolution, limitations, often correlates with oral bioavailability	Caco-2, MDCK, e.t.c
Absorption	Ability to pass the first biological barrier to entry into the body	Not as the same as the ability to reach portal blood by this definition	ROF, COMPUTATIONAL MODELS
BIOAVAILABILITY	Fraction of administrated drug that ultimately reaches the site of action, although in practice concentrations in systemic circulation are usually measured	Includes absorption and first pass effect	In vivo PK studies, computational models

#Solubility

- Importantly, two different kinds of "solubility" encountered in drug discovery need to be distinguished.
- Thermodynamic or equilibrium solubility is the true amount of compound that will go into insulin from the solid phase given an infinite amount of time. This is approximated by shaking it in a flash with the solvent for 24-72hr, filtering or centrifuging and then quantitating the amount dissolved by using HPLC standards. Obviously this is not a high-through put method.
- Kinetic solubility is typically observed when a stock solution of a compound dissolved in DMSO is diluted into water or aqueous buffer, as is done in preparing HTS samples. Although precipitation might not be observed immediately. It often happens a few hours or days later. Here a supersaturated solution is formed upon dilution, which eventually reaches equilibrium but takes some time to do so.
- In contrast to equilibrium solubility, though, kinetic solubility is easy to measure quickly, making it more suitable for high-through put determination using a method like nephelometry or turbidimetry.

Problems resulting from poor solubility

- Good solubility is important for oral bioavailability.
- Poor solubility and differences between kinetic solubilities and equilibrium solubilities can cause problem at many points in the drug discovery process.
- Poor solubility introduces uncertainty into the concentration of compounds in the wells of HTS plates.
- Different solubilities of different solid forms can make compounds hard to test and results hard to compare

Key points about solubility

- Kinetic solubility is easy to measure in high – throughput
- Thermodynamic solubility is harder to measure but ultimately more relavent
- Solubility for a given compound depends on its crystalline or amorphous form
- Inadequate solubility can
  - Make for poor absorption
  - Lead to erroneous SAR & poor compound prioritization
  - Mask other problems
- The minimum solubility a drug will need depends on its potency & permeability
- Poor solubility can be improved by
  - Reducing hydrophobicity
  - Adding polarity
  - Disrupting crystal packing interactions
  - Making appropriate salts

➢ Using a prodrug approach

Solubility leads

- In DMSO , even very insoluble drugs could be tested.
- As a result – in vitro activity could be detected in compounds with very poor thermodynamic solubility properties.
- The physico-chemical profile of leads does not depend on compound solubility.

Target dataset with good absorption properties

- Compounds that entered clinical phase 2 stage.
- Poorly soluble compounds or compounds with poorer physical and chemical properties, as well as insoluble and non-permeable compounds would have been filtered out at earlier stages.
- Data taken from world drug index(WDI) - a computerized database of about 50000 drugs.
- USAN – united nation adapted name
- INN – international non-proprietary name.
- These names are applied upon entry to phase 2
- Database size- about 2500 compounds.

Selected parameters for testing:

- Molecular weight - known relationship between poor permeability & high molecular weight.
- Lipophilicity (ratio of octanol solubility to water solubility – measured through LogP.
- Number of hydrogen bond donors and acceptors – high numbers may impair penneability across membrane bilayer.

LIPINSKI'S the rule of five

Poor absorption or permeation are more likely when:

- There are more than (5) H-bond donors.
- The molecular weight is over (500)
- The LogP is over(5).
- There are more than (10) H-bond acceptors.

Lipophilicity

Lipophilicity describes the likelihood of a molecular to participate into a lipid phase with respect to a hydrophilic phase, and to penetrate the lipid bilayer to reach the therapeutic target site.

Therefore, Lipophilicity has a wide impact on the oral absorption, distribution within the body, as well as metabolism and elimination (ADME) of drug candidates.

The terms are commonly utilized to describe Lipophilicity: LogP and LogD, referring to the logarithms of partition coefficient and distribution coefficient of new chemical entities (NCEs) in a lipophilic phase (e.g. 1-octanol) and an aqueous phase, respectively.

Specifically, the former represents the partition of neutral species only, where as LogD reflects partitions of both neutral and ionized species at a particular pH and there by pH dependent.

Charged or ionized species are less favorable for partitioning into the lipophilic phase.

Partition coefficient (PC):

The ratio of the equilibrium concentrations of a dissolved substance in a in a two-phase system containing two largely immiscible solvents(water & n-octanol

$$\text{Partition coefficient} = \frac{\text{conc. of drug in oil phase}}{\text{conc of drug in water phase}}$$

The higher the PC, the better is its lipid solubility.

Exception to the rule of five:

Compound classes that are substrates for biological transporters:

- Antibiotics.
- Fungicides – protozoacides -- antiseptics.
- Vitamins.
- Cardiac glycosides.

Isonization

Most drug candidates contain at least one ionizable group and therefore can be ionized within the physiological pH range.

Interestingly, 90% of NCEs are ionized to variable extent at different pH, leaving only 10% of them non-inonizable within pH 2-12.

At pH 7.4 at which most drugs are absorbed through gastro-intestinanes, 36% NCEs are more than 50% ionized.

Ionization is closely related to solubility and membrane permeability, thus the oral absorption of the drug.

The charge of a drug also affects the binding at the active site, thereby influencing not only CYP or hERG activity but also therapeutic efficiency.

Most frequently used techniques to improve efficiency or activity is to introduce ionizable groups to the lead or modifying the ionization of the lead.

Potentiometric titration is considered as the measurement of choice for pKa determination.

Poor solubility can be impaired by:

- Reducing hydrophobicity
- Adding polarity
- Disrupting crystal packing interactins
- Making appropriate salts
- Using a prodrug approach.

Chemical & plasma stability

- Like insolubility, compound instability can make assay results false & potentially misleading.
- Drugs need to be stable at every stage of discovery & development. Besides confounding early biochemical assay values. Instability will affect everything there after including cellular values, invivo results, a compound's ability to survive the pH range in the GI tract, & even shelflife.

Three kinds of instability often encountered are:

❖ **Oxidative instability:-**

Organic chemists are quite aware of the tendency of ether & THF to form hydro peroxides & of thiols to form disulfides given a little bit of air & a lot of time. These types of reactions usually occur in solutions by free radical mechanisms & can be catalyzed by various metal ion are light.

❖ **Chiral instability:-**

Chiral purity is important since drugs are increasingly being introduced as single stereoisomers. For drugs that are racemic to begin with the equilibration of each epimer amounts to stability.

❖ **Hydrolytic instability:-**

This can be a problem for certain functional groups like esters, amides, carbomates, sulphanamides, lactones & lactams. Hydrolytic instability for such compounds spans the

range of simple, pH dependent, non-enzymatic hydrolysis to hydrolytic cleavage of peptides by specific peptidases.

Key points about chemical & plasma stability

- Chemical & plasma stability screening can help to
  - Identify problem compounds early
  - Select compounds for invivo studies
  - Study prodrugs & antidrugs
- Stability in plasma, serum, or blood is species dependent
- Stability can be enhanced by isosteric replacement or through wide variety of modifications

Metabolic stability

Metabolically unstable compounds will tend to have poor PK properties like bioavailability & in vivo half life due to rapid degradation. Metabolic instability can also make for other problems like toxicity or drug -drug interactions caused by metabolites.

Common metabolic transformations

Xenobiotics are transformed & cleared from the body through a highly evolved system that efficiently prevents the accumulation of lipophilic & potentially toxic substances. The process involves often 2 stages(in the first, phase1 metabolism, drug's polarity is slightly increased though what's usually an oxidative or hydrolytic process like hydroxylation or ester hydrolysis. This can be followed by phase 2 metabolism, where the polarity is further increased through an enzyme-catalyzed conjugation of the resulting compound with glucuronic acid, sulphate, etc.)

Assessing Metabolic Stability

1) Recombinant Drug Metabolizing Enzymes

Individual drug metabolizing enzymes (DMEs) like CYPs, FMOs, &UGTs are now routinely expressed, usually in insect cells using a baculo virus.
The stability of a given compound could be assayed against a full collection of these individual DMEs or a cocktail there of as a primary screen for stability to Phase1 metabolism.

2) Liver Microsomes

Screening for metabolic stability using Microsomes is the most popular way of looking at a compound's stability to most, but not all important metabolic transformations. Microsomes from many different species (mouse, rat, monkey, etc) including man are commercially available & are stable for months when kept at 80° C although their

activities drop off at room temperature after an hour or two making them less useful for long time-course experiments.

3) **Liver Cytosol & S9 Sub cellular Fractions**

Hepatic Cytosol contains three important soluble DMEs that aren't found in Microsomes, N-acetyl transferase (NAC). Sulfotransferase (SULT sometimes abbreviated ST) & glutathione S-transferase (GST). When the appropriate co-factors like Acetyl CoA for NAC are added stability to these three conjugating enzymes can be studied.

4) **Hepatocytes**

Fresh primary human Hepatocytes are the gold standard for metabolism research. Advances in cryopreservation techniques have recently made it possible to store frozen human Hepatocytes for up to a year, then out & use them with only a slight loss of activity. As a result, human Hepatocytes stability screening has become widespread in drug discovery recently.

Improving Metabolic Stability

1) **Metabolic Identification**

Knowing what the compound's metabolic hotspots are, structural modifications to prevent these reactions can be carried out if the aim is to prolong in vivo exposure to the parent compound, which is normally the case except for prodrugs & ante drugs.

2) **Structural Modifications to Improve Metabolic Stability**

Site-directed methods

- Replace atoms (i.e., with a bioisostere) at the site of metabolism
- Alter electronic or steric effects to disfavor metabolism

Lipophilicity- directed method

- Reduce Log P (or Log D) by adding in polarity
  "If you can't beat 'em....." method
- Pre-form a stable active metabolite.

Key points about metabolic stability

Metabolic stability is normally essential for good PK properities.

Commonly observed metabolic reactions include the following:

- Hydrolysis of esters and amides(phase1)
- Hydroxylation of arenes and alkanes(phase1)
- Epoxidation of alkenes and alkynes(phase1)

- oxidations at heteroatom's(phase1)
- O- N- or S-GLUCURONIDATION (phase2)

Metabolic stability is typically assessed by incubation with liver Microsomes or Hepatocytes.

Fixing a metabolic stability problem might:
Cause new instability somewhere else on the molecule.
Decrease cell stability and absorption.
LEAD to faster excretion.
Turn the compound into CYP inhibitor.
Not improve stability in a different species.

Ways to increase metabolic stability include the following:

Bioisosteric replacement at the metabolic site altering electronics, sterics or conformation to disfavor metabolism. Reducing Log P by adding in polar groups pre-forming an active active metabolite.

Plasma protein Binding

Plasma protein binding reduces the amount of free drug available to the target

- It can also however, increase a compound's in vivo half life
- Many marketed drugs display high plasma protein binding.
- Many acidic compounds bind to albumin many basic ones to AAG.
- Species differences in plasma protein binding are common.

Measuring plasma protein binding:

1) Equilibrium dialysis is considered the "gold standard" method. A sample of the compound in plasma at PH7.4 and at 37_C is dialyzed through a membrane with a 10kDa cut off into buffer for some hours. After standard sample preparation, detection and quantitation of both solutions are done by LC/MS.

2) Ultra filtration - centrifugal force is applied for a few minutes to a sample of the compound in a plasma , pushing the unbound fraction through a permeability selective membrane. LC/MS analysis of the ultrafilterate and retenate is then done.

3) Computational methods - for predicting plasma protein binding have appeared in recent years, and such software is now commercially available.

Plasma protein Binding can be minimized by:

- Removing or reducing acidity.
- Adding in amines.
- Lowering Lipophilicity.
- Given enough information, using SBDD to disfavor binding.