Review Article

Role of preclinical pharmacokinetics in New Chemical Entity (NCE) development: A review

Sakshi Negi*, Ashutosh Badola*

Division of Pharmaceutical Sciences, Shri Guru Ram Rai Institute of Technology and Sciences, Patel Nagar, Dehradun-248001, India

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Abstract

The cost of bringing new chemical entity (NCE) is very expensive and long process and the chances of failure during clinical trials are very high. Inadequate pharmacokinetic data is the major problem and failure of new drug during clinical phase therefore in order to decrease drug failure rates preclinical studies such as *in vitro* and *in vivo* pharmacokinetic studies are being conducted. At present, most pharmaceutical companies perform *in vitro* and *in vivo* studies together with *in silico* prediction softwares. The data obtained during preclinical phase helps in eliminating weaker candidate and reduce attrition rate. The aim of this review is to focus on various methods employed during preclinical phase.

Keywords: preclinical, *in vitro, in vivo* research, pharmacokinetics, new chemical entity

Introduction

Developing a new compound with desired therapeutic activity required large amount of resource, time and energy therefore to identify a new drug with favorable potency is a cumbersome process. The process of delivering a new drug from discovery to human use consists of multiple stages such as target selection, lead selection and optimization, preclinical studies and clinical research (Prentis, 1988).

The process of identifying a new compound consists of two steps i.e. (shown in figure. 1)

- Drug development
- Drug discovery

Drug development

Before developing a new drug it is important to understand diseases its mechanisms and researchers need to find the causes and possible treatment of diseases so the discovery of new drug (new chemical entity NCE) include identification of a specific target sites for a particular diseases and developing various *in-vitro* and *in-vivo* tests to discover possible treatment against diseases (Wang and Urban, 2004).

Address for Corresponding Author:
Sakshi Negi
Division of Pharmaceutical Sciences, Shri Guru Ram Rai Institute Of Technology And Sciences, Patel Nagar, Dehradun-248001, India.
Email: negisakshi03@gmail.com

Drug discovery

After screening and optimization of various hits compounds, one or two lead compounds are optimized and validate. Scientists, researchers conduct various studies. These studies include: preclinical and clinical study.

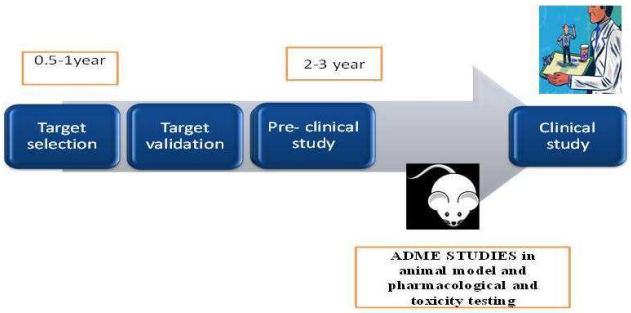

Figure.1 Schematic diagram of steps involved in drug development and discovery

Preclinical studies

Discovery of NCE is not a simple process as discussed above and encompasses of multiple disciplines and not every discovery results in development of potent new drug sometimes it fails to show any pharmacological activity and main reason of this failure is insufficient pharmacokinetic parameters and metabolic studies (Agrawal, 2015). Therefore Preclinical studies came into light to overcome from these limitations. Before conducting clinical trials researchers make sure that the optimized lead compound has

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sufficient metabolic and pK parameters. Preclinical studies are carried out to establish various parameters such as pharmacokinetics parameters, toxicity levels, and therapeutic index. Preclinical studies are carried out to ensure that the new drug (NCE) has a potent therapeutic activity and it does not causes any toxicity or other harmful effects on humans therefore preclinical studies are carried out on animal models (such as mice, rats ,hamster, monkey etc) under laboratory conditions (Zambon, 2015).

***In-vitro* studies**

All the experiments are carried out in research lab under controlled environment. *In-vitro* and *in-vivo* studies in animals are necessary to performed so to established pharmacokinetics parameters. These studies are helpful to understand interspecies differences. There are several studies which play an essential rule during preclinical research of any new drug. It helps in establishing various parameters which can help scientist in selecting a desired candidate and reducing failures. Following are the some important parameters which can be used to characterize the new drug physiochemical properties (Xiong, 2015).

Solubility

Solubility is a dynamic process which can be defined as maximum amount of solute dissolved in a given solvent to form a homogenous solution.

IUPAC defines solubility as the analytical composition of a saturated solution expressed as a proportion of a designated solute in a designated solvent. Solubility may be stated in units of concentration, molality, mole fraction, mole ratio and other units. Many new drug fail during preclinical stages due to poor bioavailability therefore it is essential to determine solubility of new chemical entity (NCE) (Den Haan, 2015).

Importance of solubility during preclinical development of new drug

The major challenge occurs during the discovery and development of a new drug is low solubility of new compound. Low solubility affects pharmacokinetic and dynamic properties of the compound and therefore affecting *in vitro* and *in vivo* performance of new compound during drug discovery and therefore causes high risk of failure (Chen et al., 2009).

Low aqueous solubility also causes major problem during development of new drug well as generic development. To achieve maximum absorption, the compound should be present at the site where absorption takes place. More than 40% of NCEs developed in the pharmaceutical industry are insoluble in water. For this reason, the problem of solubility is one of the major challenges for formulation chemists. It is also important to determine solubility during drug discovery in respect to target-based assays. Solubility can be estimated by two methods i.e. thermodynamic and kinetic method (Mankowski et al., 2003).

***In-vitro* stimulated gastric fluid (SGF) stability studies**

For oral administration of drug it should be completely absorbed from the gastrointestinal tract (GIT) therefore the drug should remain stable in GIT. It is observed that many drugs have low bioavailability as they are degraded in stomach at low pH (1-2) or intestine (6-8). Simulated gastric fluid (SGF)/Simulated Intestinal Fluid (SIF) mimic GIT in terms of acidity/basicity are a perfect media to determine the stability of the drug candidate *in-vitro*. For any new chemical entity (NCE) it should be evaluated for in vitro stability study to examine any degradation during oral pharmacokinetic studies.

Plasma stability

When selecting a new chemical entity for further screening testing such as in-vivo research it is essential that the new drug must be active enough to reach the desired target and show therapeutic effect therefore in order to do so in vitro assays including plasma stability assays are carried out during early phase of preclinical research. Plasma stability assays provide information in estimating the degradation of compound in plasma. This assay helps scientists and researchers in screening out prodrugs and antedrugs, identifying the compounds which are liable to hydrolyse in plasma: esters, amides, lactones, lactams, carbamides, sulphonamides, and peptic mimetics. Stability of NCE in matrix such as plasma play major role in maintaining the desired drug plasma concentration and half life to show therapeutic effect. Drugs which are unstable in plasma are liable to have shorter half life as they are rapidly cleared out from the body and therefore exhibits unstable pk profile as the drug tend to degrade during sampling of blood collection. Therefore plasma stability assays helps in screening of potent lead molecule for further in vivo studies (Reichel et al., 2015).

***In-vitro* metabolic study**

Drug metabolism is defined as process where drugs are converted to water soluble compounds or metabolites via various enzymatic reactions so to excrete out from the body. So it is important to know the rate of drug metabolism as it helps in the prediction of various pharmacokinetic parameters in humans such as oral bioavailability (Caldwell, 2015). The *in-vitro* metabolic comprise of microsomal stability of the drug and CYP450 reaction phenotyping (Ghanbari et al., 2015). These studies are usually performed on liver microsomes of animal's model.

Liver microsomes are the subcellular fractions which consists of various metabolic enzymes such as cytochrome P450, carboxylesterases etc. conversion of drug into its water soluble compounds consists of 2 phases i.e. phase I and II. Phase I reaction involves oxidation, reduction and hydrolysis reaction and CYP-P450 and flavin-containing monooxygenases (FMOs) are the enzymes which act as a catalyst in the reaction. Where as in phase II reaction involve additions (or conjugations) of highly polar groups to the molecule and the enzymes involved in the conversions is UDP-glucuronyltransferases (UGTs), sulfotransferases. *In-vitro* metabolic stability helps in predicting various parameters such as half life, intrinsic clearance and hepatic clearance (Rendic et al., 2002).

Importance of microsomal stability in preclinical study of new drug

Potent drug has to stay in the human body for an adequate length of time to interact with the intended pharmacological targets using a reasonable dose regiment. The duration of a drug in the body is mainly determined by hepatic metabolism, hepatic excretion and renal excretion. In vitro metabolic assay is used to identify for chemical structures with the most appropriate stability upon hepatic metabolism – metabolic stability. The rate of drug metabolism helps in predicting the pharmacokinetics parameters such as oral bioavailability, clearance and half-life. This helps in determining the efficacy and toxicology of the drug For example, a drug that is rapidly metabolised may require multiple daily dosing or higher doses to maintain a concentration in the bloodstream or target organ that is sufficient to elicit a therapeutic effect. However, very slowly metabolised drugs may also cause issues if they remain in the body for long periods, causing accumulation of the drug and potential toxicity. Metabolic stability of a new drug is performed on the bases of drug disappearance during incubation of microsomes (liver). Based on the results half live and intrinsic clearance is calculated.

Metabolic stability of NCE is determined based on disappearance of the test drug during incubation with human/animal liver fractions, and metabolic stability provide various parameters such as *in vitro* half-life ($t_{1/2}$) and intrinsic clearance (CL_{int}). Based on these values secondary pharmacokinetic parameters, such as hepatic clearance (CL_H), bioavailability and *in vivo* $t_{1/2}$ can be calculated. If a test compound is rapidly metabolized, its bioavailability *in vivo* will probably be low. Therefore, only compounds with expected suitable pharmacokinetic properties are chosen for further development (Tukey et al., 2010).

Plasma protein binding

Drugs molecules are either bound to proteins and lipids or present in free form. Interspecies differences in plasma protein binding (PPB) can lead to a reduced or increased drug-safety margin [40]. it is highly recommended that PPB should be determined in animal plasma before entering to clinical phase trials therefore PPB should be determined during preclinical phase as it provide useful data for determining drug- drug interaction(Zhang et al., 2012).

Plasma protein-binding affects various pharmacokinetic parameters such as plasma clearance, elimination half-life, apparent volume of the distribution, and area under the curve.

Techniques used in ppb

There are several methods which are used to separate the bound and unbound fraction of drug molecule from plasma. These include ultrafiltration, ultracentrifugation and equilibrium dialysis. Equilibrium dialysis is the most commonly used method. However, if a drug or a metabolite is not stable in plasma for 3–5 h, this technique cannot be used and ultrafiltration might be a better alternative. Ultracentrifugation requires long centrifugation times. This technique is rather expensive and is not commonly used (Lee et al., 2013). During drug discovery, equilibrium dialysis, ultrafiltration, ultracentrifugation and Transil™ partitioning are all used for separation of bound and unbound drugs. In general, PPB studies are conducted in triplicate at one standard drug concentration ranging from approximately 0.3 to 10 μM. Some companies perform more elaborate experiments, using two or three different drug concentration levels with one to five replicates at each level. In very early drug discovery 'PPB screening' is conducted by approximately 50% of the responders. A minority of the responders evaluate the mass balance/recovery using an acceptance criterion of 80%.

***In-vivo* pharmacokinetic param eters**

In-vivo pharmacokinetic (PK) screening helps in selecting lead compound and provide toxic and therapeutic efficacy of NCE. For in vivo pk studies animal models such as mice, rats, hamster or monkeys are employed to generate in- vivo pk profiling which provide useful parameters such as Bioavailability(F), Area under curve(AUC), Half life ($t1/2$),Volume of distribution (v_d),Drug clearance, C_{max}, $t_{max.}$

With sophisticated technology such as high-throughput liquid chromatography-mass spectrometry (LC/MS/MS) bioanalysis, makes work easier for researchers to generate results helpful in selecting new drug (Urso et al., 2002). C_{max}, $t_{max.}$ $t_{1/2}$ and AUC are first parameters which are estimated during the analysis of pk data as these parameters doesn't required any complicated mathematical model. These data can be obtained simple graphical method by plotting plasma concentration with time.

C_{max} and T_{max}: it represents maximum plasma concentration and time respectively which can be observed directly by graphical representation.

AUC: area under curve of any new drug helps in estimation of other important pharmacokinetic parameters such as clearance, bioavailability etc. AUC can be determined by trapezoidal method when blood, plasma (most commonly obtained), or serum drug concentrations are plotted versus time, the AUC is the primary measure of overall exposure following i.v. or extravascular administration of a drug.

$t_{1/2}$: it is defined as the time taken to reduce the concentration of drug to half of its original value in plasma or 50% from its total amount. Half life is an important parameters as it decide how long drug will stay in the body, shorter the half life more will be the dosing frequency and larger the half life drug remain in body for longer period of time which may cause toxic effects therefore half life is an essential parameter which help a pharmacist in designing the dosages regimen (Toutain, et al.,2004).

Half-life ($t_{1/2}$) of a drug, volume of distribution and clearance of drug is related according to the following equation:

$$T_{1/2} = 0.693 \times (Vd / Cl)$$

Thus the half-life of a drug is a derived parameter from Cl and Vd, Half-life of a drug is function of its blood and tissue binding as well as its total clearance (Dundee, et al., 1986).

Bioavailability: bioavailability is defined as rate and extent of active pharmaceutical ingrident or active molecule from its dosage form gets absorbed and reaches to site of action (Benet, et al., 1996).

Clearance: it is a hypothetical term which is defines as volume of blood/plasma that should be eliminated of drug per unit time whenever it passed from eliminating organ. It is a pharmacokinetic term which depicts pathway of drug elimination from the body. Drug clearance (*body clearance, total body clearance*, or Cl_T) considers the entire body as a single drug-eliminating system from which many unidentified elimination processes may occur. Drug clearance is described in terms of volume of fluid clear of drug per time unit (mL/min) [Choi, et al., 2007]. Clearance can be expressed by following equation:

$$Clearance\ (CL_T) = \frac{elimination\ rate}{plasma\ concentration}$$

Volume of distribution: it is a hypothetical term which can be defined as amount of drug in body to the concentration of drug in plasma, blood and unbound in tissues.

Lipophilic drugs have larger volume of distribution as drugs are largerly distributed throughout the body while hydrophilic drugs have smaller volume of distribution.

Pharmacokinetics

Pharmacokinetics (PK) is defined as study of body process affecting the movement of xenobiotics compounds (drug/food/NCE). Pharmacokinetics studies deals with four fundamental processes which influence the in vivo PK of a drug or NCE are absorption, distribution, metabolism and excretion (ADME). The Absorption, distribution, metabolism, and excretion are collectively called as ADME processes. ADME of a drug can be explained as when a drug is administered via oral route it first disintegrate and reaches to sites of action where the **absorption** of drug/NCE takes place after getting absorbed it enters to blood and reaches to tissues and intracellular fluid and get binds to various receptor this process is called as **distribution** while some drug molecules binds to receptor some molecules again enters to blood stream where some biochemical changes occur simply called as **metabolism (liver or other organs)** and remaining drug and metabolites are eliminated from the body via urine, feces or by any other routes. Metabolism and excretion together is pathway for **elimination**.

Absorption

Absorption refers to the movement of drug from its site of action to blood stream. It is also defined as rate and extent of drug absorbed from its sites of action upon administration of drug orally. Peroral route is the most common and important sites for the absorption of drug but absorption through the skin, the cutaneous tissue, the nasal epithelium, the peritoneum or the respiratory tract would need to be considered for dermally, subcutaneously (s.c.), intranasal (i.n.) or intraperitoneally (i.p.) administered or inhaled drugs, respectively. To get absorbed from blood stream it is important for a drug or compound to permeate from the membrane regardless any route of drug absorption.

When a drug is administered orally (such as via tablet and capsules) it must be dissolved before absorption takes place and the process of dissolving of a drug is called **dissolution**. Tablet disintegrate into smaller particles in order to get dissolve after dissolution the drug particles are reached to site of action through blood stream.

Factors affecting dissolution rate are:

Manufacturing processes and water solubility of drugs: lipophilic drugs dissolve slowly into gastric fluid while hydrophilic drugs are readily dissolves in gastric fluid.

Particle size: smaller particle size dissolve more because of large surface area and they easily break into smaller particles therefore more is the dissolution rates.

Formulations: different types of formulation affects dissolution rates sustained release tablets or gastro retentive

formulation delay the dissolution rates while fast disintegrating tablets dissolve at faster rate while liquid preparations such as syrups doesn't require any dissolution therefore have fastest dissolution rates.

After dissolution, drug molecules have to pass from the cell membrane of the GIT in order to reach blood stream. Two types of mechanism have been illustrated for the transportation of drug across the cell membrane i.e. **passive diffusion and active transport.** Passive diffusion occurs when there is a high concentration of the drug on one side of the membrane and a low concentration on the other side. This difference from one side of the membrane to the other is called a concentration gradient. Therefore it is natural that substances move from higher concentration to lower concentration. Drug molecules move across membranes or move through pores between the epithelial cells. So particles with small sizes are easily transported through passive diffusion but compounds with high molecular weight cannot be transported through passive diffusion. A few drugs that closely resemble naturally occurring compounds are absorbed via carrier-mediated transport. This process requires carrier proteins that attach to and actively carry the drug molecules across the membrane, utilizing a natural "pump" mechanism. This method of absorption is limited by the availability of the carrier protein and is therefore, saturable. Carrier-mediated transport requires energy and can move molecules against the concentration gradient.

Distribution

After getting absorbed from the bloodstream, the drug molecules are distributed throughout the body through systemic circulation. This process is called distribution, and is a reversible process; some drug molecules may bind to the receptor sites on the cell membranes or may move back to blood stream or may go back to other tissues. The delivery of a drug from the bloodstream to the site of drug action primarily depends on drug's physiochemical properties or patients conditions such as blood flow, organ perfusion, capillary permeability, the degree of binding (attachment) of the drug to blood and tissue proteins, and the relative lipid-solubility of the drug molecule. Drug molecules are distributed to eliminating organs, such as the liver and kidney, and to non-eliminating tissues, such as the brain, skin, and muscle. In pregnancy, drugs cross the placenta and may affect the developing fetus. Drugs can also be secreted in milk via the mammillary glands, into the saliva and into other secretory pathways. A substantial portion of the drug may be bound to proteins in the plasma and/or in the tissues (Lin, et al., 2003).

Metabolism

Metabolism is the major pathway of elimination for many drugs and new drug. Liver plays major role in the elimination of many xenobiotics compounds however other extra hepatic organ also have significant role in elimination of many compounds. Metabolism can be defined as biotransformation of lipophilic compounds into hydrophilic compounds or metabolites so it can be easily eliminate or excrete out from the body in form of urine or bile. Metabolism reactions are divided into two categories: Phase I and Phase II reactions [Rowland et al., 1995].

Phase I: involve oxidation, reduction and hydrolysis. The major Phase I enzyme families include the cytochrome P450 (CYP) superfamily, the flavin-containing mono-oxygenases (FMO), the monoamine oxidases, alcohol or aldehyde dehydrogenases, reductases, esterases, amidases and epoxide hydrolases.

Phase II: it involve additions (or conjugations) of highly polar groups to the molecule. These can be sequential to Phase I reactions, for example a compound may first be hydroxylated (Phase I reaction) and then the same hydroxyl group may be conjugated to glucuronic acid (Phase II reaction). However, Phase I reactions do not always precede Phase II reactions and occasionally, direct Phase II reactions occur if susceptible functional groups are present on the molecule. Common Phase II reactions include glucuronidation, sulphation, methylation, N-acetylation and glutathione conjugation (Venkatakrishnan et al., 2003).

Various factors affect metabolism such as:

Drug- drug interaction: when two or more than two drugs are administered at the same time and affect the metabolism of parent drug it is called as drug-drug interaction. It may result on increase or decrease of the parent drug resulting in low therapeutic effect or toxic metabolites.

Genetic polymorphisms: variations in metabolism in different genetic population can be explained in some cases for e.g. many Asians and Native Americans have difficulty metabolizing drugs that require acetylation, such as ethanol. These individuals will exhibit a low tolerance of such drugs, and can suffer adverse drug reactions at a much higher rate than the average population.

Age: age is another factor which affects the rate of metabolism in different age groups such as in pediatric patient organs are not fully developed whereas in geriatric patient the functioning of organs start declining therefore impacting differences in in binding proteins, drug metabolising enzymes and/or drug transporters and renal filtration/secretion which can impact on the pharmacokinetics of a drug.

Impaired renal and hepatic function: in diseased state such as in chronic renal failure kidney is unable to function properly therefore affecting clearance of drug from body

affecting plasma levels of drugs thus causing adverse effects.

Elimination

After getting distributed throughout in the body, the drug must be eliminated from the body as frequent dosing without elimination results in toxic effects of drug in body. Therefore elimination can be defined as the removal of entire drug molecule from the body. Elimination consists of both metabolism and excretion process. The 2 major pathway of elimination are: renal and biliary excretion. Renal excretion usually involves one or more of three distinct processes: glomerular filtration, tubular secretion and reabsorption from the renal tubular lumen. Biliary excretion, a process that is often facilitated by active transport systems located in the canalicular membrane of the hepatocyte, can be an important hepatic elimination pathway for many compounds. The kidneys act as a filter for the blood and create urine as a vehicle for removal of waste. Blood enters the kidney through renal arteries and then is filtered by the glomerulu. The glomerular filtrate becomes concentrated and substances are removed as it passes through the renal tubule and eventually becomes urine. Drug molecules in the bloodstream that are not bound to albumin are also filtered out into the glomerular filtrate. When drugs have not been converted to water soluble compounds in the liver, they are likely to be reabsorbed back into the bloodstream at the end of the filtration process, and will cycle through the body again. If they are water soluble, they will end up in the urine and be excreted.

Role of preclinical pharmacokinetics in drug development

Earlier during the discovery and development of NCE the main focus was on selection of most potent compound based on *in vitro* data which often failed to generate data whether the drug has a potency or have desired therapeutic effects and to reach its desired sites. According to U.K. owned pharmaceutical companies a survey was conducted up to 1985 revealing that 39% of NCEs failed in the clinic due to poor PK properties (Kumar et al., 2001).

The average cost of a new chemical entity (NCE) was approximately 900 million USD and average time taken by NCE from development till IND registration was 12 years. In 2002 the US Food and Drug Administration conducted a survey and found out that only 17 NCEs were approved which was the lowest approval rate in the past decade and in 1991 it was found that lack of sufficient PK data was the main reason for the failure of NCE .As Hodgson has cogently noted –'A chemical cannot be a drug, no matter how active nor how specific its action, unless it is also taken appropriately into the body (absorption), distributed to the right parts of the body, metabolized in a way that does not instantly remove its activity, and eliminated in a suitable manner – a compound must get in, move about, hang around, and then get out'. Thus, when analyzing properties of a drug, especially an NCE, in vivo without knowledge of its PK properties – even at discovery level – is an exercise in futility. The potent lead compound discovered after drug discovery are examined during preclinical studies. Preclinical examination is the second step in the drug development process. The role of preclinical examination is the identification of pharmacological properties like the mode of action (pharmacodynamic) and the metabolism (pharmacokinetic) of a substance. Furthermore, pharmacological testing should lead to an extrapolation of animal data to humans. This studies help in establishing pharmacological properties of new compound and provide information about adverse and toxic effects of a new compound before entering to clinical phase, therefore efficacy and toxicity studies are integral part of preclinical studies but pharmacokinetic (PK) studies in animals have also become a important part of preclinical research during drug development. Before development and validation of in vitro studies PK studies in animals were used routinely. Therefore preclinical pharmacokinetic parameters play important role during drug discovery (Kola and Landis, 2004).

Conclusion

Preclinical pharmacokinetic plays a vital role in the development of new drug. It reduces the chances of drug failure and in turn reduces the cost and timing. With the use of sophisticated instrument and advanced technology it become easier to predict early ADME Parameters and eliminating weaker candidates and selecting lead candidate for clinical purpose in humans**.**

Conflicts of interest: None

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