IJCRT.ORG ISSN : 2320-2882

INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

Biopharmaceutics And Pharmacokinetics In Drug Research

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Abstract

With the synergistic and multiplicative interactions of rational drug design, recombinant biotechnology, combinatorial chemistry and high-throughput screening, millions of compounds are being synthesized by chemists. However, development of these drug candidates has often been impeded, if not terminated, due to biopharmaceutic and/or pharmacokinetic constraints. This has resulted in delays in development time and escalation of cost in the drug research programmes. So, the present emphasis is to reduce development time and cost, which is analogous to added patent life besides the enormous reduction in human suffering. In this compilation the important biopharmaceutic and pharmacokinetic approaches are discussed, which will help in the development of safe and more efficacious drugs with reduced development time and cost.

***Keywords*:** New drug development; Biopharmaceutics; Pharmacokinetics; Solubility; Permeability; Lipophilicity

1. Introduction

Drug research is a unique multi-disciplinary process heading towards the development of novel therapeutic agents in areas of currently unmet medical need. The drug research can be divided functionally into two stages: discovery/design and development (Fig. 1). Drug discovery/design consists of identification and characterization of new targets (enzymes or receptors), synthesis of new lead molecules, screening of new lead molecules for in vitro and/or in vivo biological activities, and physicochemical characterization of leads. Drug development focuses on evaluation of safety/toxicity and efficacy of new drug molecules. The key objective of drug development is the generation of a scientific database that supports the effectiveness and safety profile of the dosage regimen(s) intended for marketing. On an average every new drug molecule requires 12–15 years to reach the patient and costs a staggering amount of US$ 400–650 million (Collins et al., 1999).

Although new methods, such as pharmacophore theory and molecular modeling, are be-

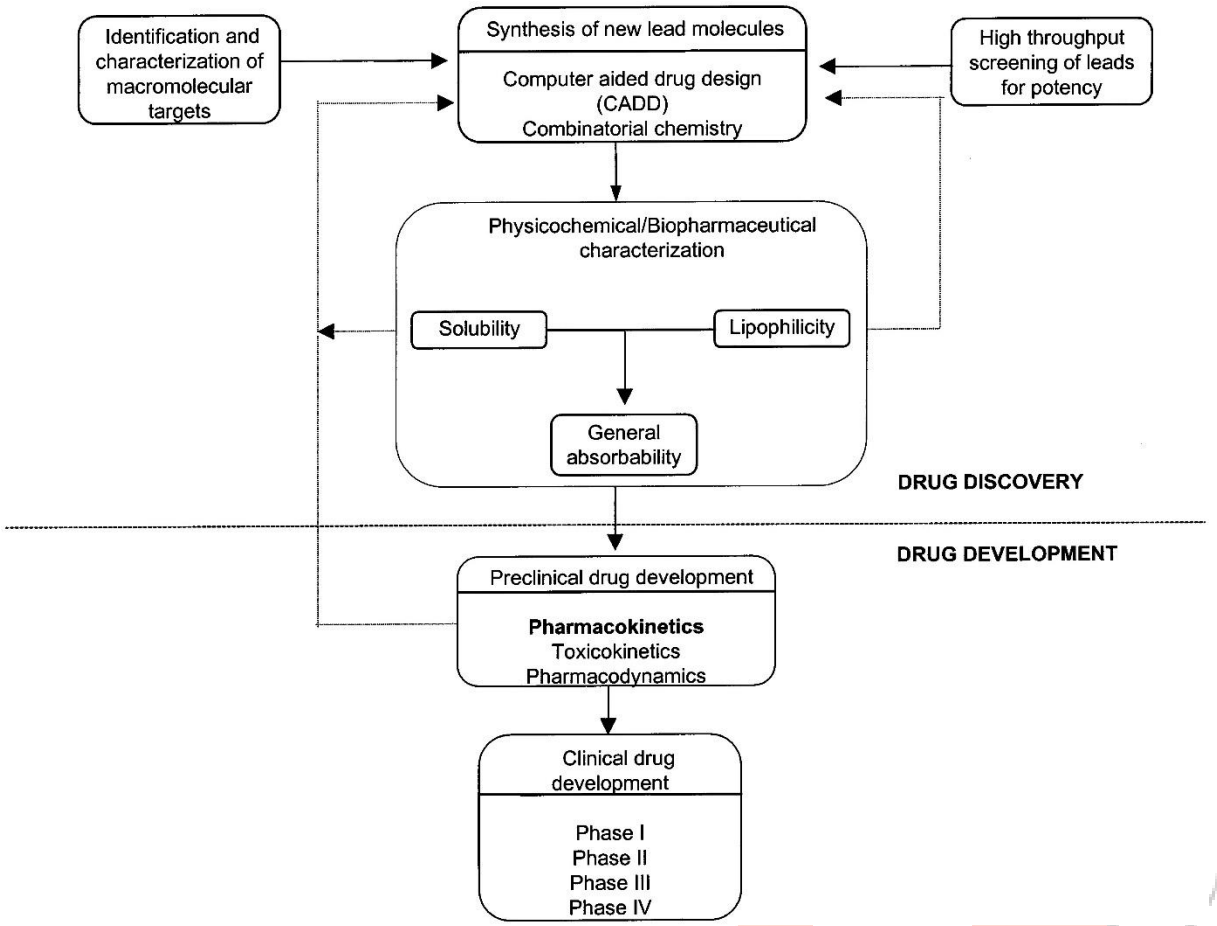

Fig. 1. Various stages of drug research.

ing used to assist rational drug design, the classical method of choosing potential drugs for drug development in the pharmaceutical industry still relies on an evaluation of pharmacodynamic activity in vitro and/or in animal models. However, majority of the drug molecules fail in subsequent drug development program because the efficacy and safety are not governed by its pharmacodynamic characteristics alone. It also depends to a large degree on the biopharmaceutical (e.g. solubility, stability, permeability and first pass effect) and pharmacokinetic (clearance rate, biological half-life, extent of protein binding and volume of distribution) properties of the drug, since these properties control the rate and the extent to which the drug can reach its site of action, i.e. biophase.

Some data on reasons for withdrawal of candidate drugs from development has been published by the Center for Medicines
Research (Prentis et al., 1988), which says that nearly 40% of the drugs (78 of 198 compounds) were terminated from further development due to unsatisfactory pharmacokinetics (Table 1).

Table 1
Reasons for failure in drug development (data obtained from
Prentis et al., 1988) (%)

Poor biopharmaceutical properties	40
Lack of efficacy	30
Toxicity	21
Commercial reasons	8

Whereas poor pharmacokinetics were solely responsible for nearly all (90%) terminations of anti-infective drugs. Hence understanding of these properties is essential to all scientists involved in drug discovery and development.

2. Events that lead to in vivo drug action

To elicit in vivo response, the drug must reach the biophase, crossing several biomembranes. Many factors are responsible for the entry of a drug into the body and then into biophase. These factors include the route of administration; the dosage form; the liberation rate of the drug from the dosage form; dissolution; penetration and permeation of the drug through biomembranes; its distribution within the body fluids and tissues; the type, amount and rate of biotransformation; and recycling processes and elimination. In addition to these factors, pharmacogenetics and certain pathophysiological conditions also affect the above process. The entire process can be described as the LADMER (liberation, absorption, distribution, metabolism, elimination and response) system showing that liberation, absorption, distribution, metabolism and elimination are scheme shown in Table 2 (Ritschel, 1992b).

3. Biopharmaceutics in drug development

Biopharmaceutics is a major branch of the pharmaceutical sciences concerned with the relationship between the physicochemical properties of a drug in dosage form and the pharmacologic, toxicologic, or clinical response observed after its administration (Gibaldi, 1991). In short, biopharmaceutics mainly involves drug absorption process. Systemic absorption of most drug products consists of a succession of rate processes viz.: (i) disintegration of the product; (ii) dissolution of the drug in an aqueous environment; and (iii) absorption across cell membranes into the systemic circulation and, ultimately, to its site of action. Several key hurdles such as aqueous solubility, stability, permeability, and first pass effect, must be overcome so as to get a drug from a dosage form to its site of action (Fig. 2). These hurdles vary in how easily they can be overcome by formulation efforts (Lipper, 1999). When it becomes evident that a potential drug candidate has poor biopharmaceutic properties, due consideration must be given to its developability.

involved to elicit the response (Ritschel, 1992a). One can subdivide this approach according to the

Several mathematical models have been proposed (Goodacre and Murray, 1981; Ho et al., 1983; Dressman et al., 1985; Dressman and Fleisher, 1986; Macheras and Symillides, 1989; Sinko et al., 1991; Oh et al., 1993) to estimate oral absorption/bioavailability. A simple dimensionless number, absorption potential (AP) proposed by Dressman et al. (1985) appears to correlate quantitatively with fraction absorbed (Macheras and Symillides, 1989) as well as identify the critical limiting physicochemical property of poorly absorbed compounds. However, as the AP is mainly concerned with the physicochemical properties of the drug it cannot be used as the sole indicator of bioavailability. Whereas, the mathematical models of Sinko et al. (1991) and Oh et al. (1993) demonstrated good correlations between in vitro dissolution and in

vivo bioavailability. These models point out that the key parameters controlling drug absorption are three dimensionless numbers; an absorption number (A_n), a dissolution number (D_n) and dose number (D_o); representing the fundamental processes of membrane permeation, drug dissolution and dose, respectively (Amidon et al., 1995). By calculating all these dimensionless numbers for a new molecule and by referencing in contour plots (Oh et al., 1993), an estimate of the extent of drug absorption can be made. However, all these numbers are related to two important parameters controlling drug absorption, i.e. solubility and permeability (Amidon et al., 1995). Based on these two parameters Biopharmaceutic Drug Classification Scheme (BCS) has been proposed by Amidon et al. (1995), which happens to be an important tool in developing a suitable strategy for improving the bioavailability of new chemical entities (Fig. 2). If formulation efforts can overcome the biopharmaceutic hurdle without consuming much of time and resources, one may go ahead with formulation modifications. Chemical modification of structure of the lead compound is the other strategy to overcome biopharmaceutical hurdles.

As a result of combinatorial chemistry and high throughput screening millions of compounds are getting added to the library. In selecting the right candidates from the database, for further development, many major pharmaceutical companies have taken novel approaches that consider biopharmaceutic properties like solubility, permeability and other physicochemical parameters. In this section more emphasis is given to important biopharmaceutic parameters that affect its absorption and/or bioavailability. The various biopharmaceutic factors affecting dissolution, absorption and/or bioavailability of drugs are listed in Table 3.

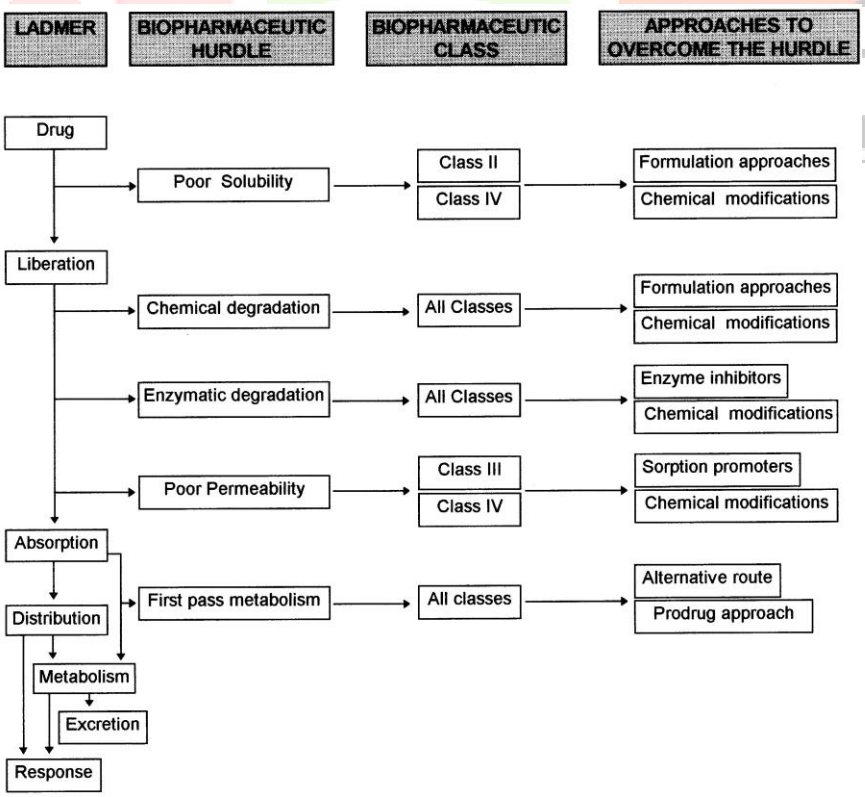

Fig. 2. LADMER system, biopharmaceutic hurdles in drug development approaches to overcome them.

Table 3
Biopharmaceutic factors affecting dissolution and/or absorption

Physicochemical characteristics of the drug
Solubility
Partition coefficient, pK_a
Dissolution rate
Salt formation
Prodrugs
Particle size, surface area and shape
Crystallinity, polymorphism
Stereochemical factors
Pharmaceutical factors
Factors related to formulation (excipients)
Galenical/dosage form
Manufacturing process
Stability and storage of drug and drug product
Physiological factors affecting bioa6ailability
Permeability
GI transit/motility
Site specific absorption
Preabsorptive metabolism
Hepatic metabolism
Biliary excretion
Renal excretion
Protein and tissue binding

3.1. *Solubility*

Solubility is an important determinant in drug liberation and absorption and hence plays a key role in its bioavailability. For a drug to be absorbed it must be present in the form of an aqueous solution at the site of absorption. This is true regardless of the mechanism of absorption, whether it be passive diffusion, convective transport, active transport, facilitated transport or ion pair transport, except in the case of pinocytosis, which is very rare and applies only to the fat-soluble vitamins A, D, E, K, and some lipids. If the drug is insoluble or very poorly soluble it poses a problem of dissolution and/or absorption, since the flux of drug across an intestinal membrane is proportional to its concentration gradient between the apical side and basolateral sides of GI lumen. Hence, the aqueous solubility of drugs is of central importance in the process of drug discovery and development, from molecular design to pharmaceutical formulation and biopharmaceutics.

The aqueous solubility of solid compounds is governed by interactions between molecules in the crystal lattice, intermolecular interactions in the solution, and the entropy changes accompanying fusion and dissolution. The observed mole fraction solubility of any solute X_w is related to its ideal mole fraction solubility, X_i, and the activity coefficient in water, g_w, as shown in Eq. (1).

$$\log X_w = \log X_i - \log g_w \quad (1)$$

Eq. (1) clearly says that both crystalline structure as well as the activity coefficient can contribute to the insolubility of a solute (Yalkowsky, 1981). Therefore, solubility of poorly soluble solute can be increased by two approaches. One is by increasing its ideal solubility by chemical modifications or solid state manipulations, and the second approach is to decrease its activity coefficient to unity by formulation changes. First approach includes introduction of polar or ionizable groups or introduction of groups that decrease melting point (Amidon, 1981) or polymorphic alterations. The second approach includes micellar solubilization, cosolvency, hydrotrophy, and formation of inclusion complexes.

The development of various peptidomimetics like HIV protease inhibitors and fibrinogen receptor (GP IIb/IIIa) antagonists are good examples that illustrate the concept and importance of drug solubility in drug selection and drug development. A variety of compounds that display excellent inhibition of HIV-protease have been designed by molecular modeling. However, most of these retain peptidic nature (Bohacek et al., 1996) and hence, have poor biopharmaceutic and pharmacokinetic properties such as low aqueous solubility/high lipophilicity, high molecular weight, susceptibility to proteolytic degradation, high hepatic metabolism and biliary

excretion, characterized by low oral absorption and rapid elimination (Kempf et al., 1995). The identification of HIV protease inhibitors with optimal oral bioavailability, high potency and selectivity therefore represents a critical milestone. In order to improve oral bioavailability, it may be necessary to change physicochemical properties such as solubility by designing modifications in non-pharmacophore regions (first approach). The following examples best illustrate this point.

Vacca et al. (1991) initially developed a series of hydroxyethylene dipeptide isosteres, represented by L-685,434, and were found to be highly potent and selective HIV protease inhibitors. Although they are highly potent and selective, the main drawback was that they lacked aqueous solubility and an acceptable pharmacokinetic profile, resulting in poor bioavailability (Thompson et al., 1992). The efforts made to increase the solubility by incorporating a basic amine (replacement of *tert*-butyl carbamate and Phe moieties with decahydroisoquinoline *tert*-butylamide) into the backbone of this series, led to the development of a novel class of hydroxylamine pentanamide (HAPA) isosteres, represented by L-704,486, with a favorable oral pharmacokinetics but its efficacy was diminished. When decahydroisoquinoline *tert*-butylamide group was replaced with two *tert*butyl carboxamide 4-substituted piperazines, the basic amine improved aqueous solubility and N4 gave a chance for further modifications that could balance hydrophilic and hydrophobic requirements. The 3-pyridyl methyl substitution at N4 (lead to the discovery of L-735,524/Indinavir) provided both lipophilicity for binding to the target and a weakly basic nitrogen further increased aqueous solubility (Fig. 3). Indinavir sulfate is the clinical formulation, because of improved aqueous solubility (\450 mg/ml) and consistent bioavailability (Dorsey et al., 1994). Development of ritonavir is another such example where solubility approach had been applied in the drug development (Kempf et al., 1991, 1995). Another illustrative example was reported by Eldred et al. (1994). They synthesized a series of benzamidine containing fibrinogen receptor (GP IIb/IIIa) antagonists. But the highly potent compounds when dosed orally to marmoset showed very low oral activity and was assumed to be due to poor absorption that in turn was claimed to be the result of their poor aqueous solubility. When the solubility of these compounds was increased by introducing piperazine ring, the oral activity was improved attesting the importance of good aqueous solubility for drug absorption.

One of the techniques commonly used to overcome the problems of poor and erratic bioavailability is the prodrug approach, wherein the physicochemical properties of the drug are improved by bioreversible chemical alteration. The most common prodrug strategy involves the incorporation of a polar or ionizable moiety into

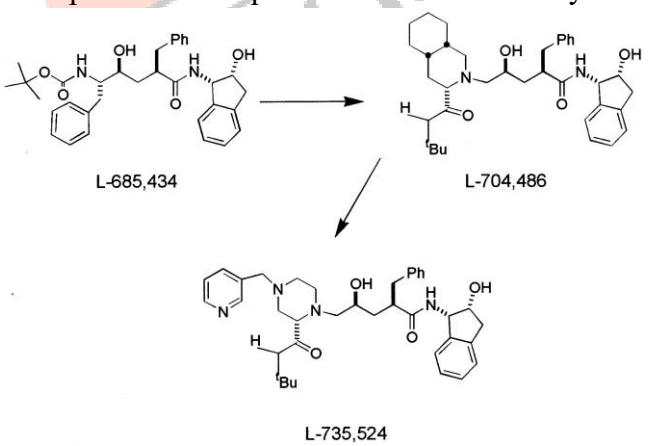

Fig. 3. Chemical structures of L-685,434, L-704,486, and indinavir.

the parent compound to improve aqueous solubility (Stella et al., 1998). In 'ad hoc prodrug approach' the target activity is optimized first irrespective of pharmacokinetic properties and once the target activity is demonstrated, the pharmacokinetic properties are optimized by prodrug design without altering the pharmacophore in the active chemical (Testa and Caldwell, 1996). Such an approach may well prove most useful or even indispensable in the development of therapeutic peptides and antisense drugs. Though the application of this approach in drug research is uncommon, it was successfully applied in the development of enalapril, an angiotensin converting enzyme inhibitor. The 'post hoc prodrug approach' (prodrugs of established drugs) has been successfully used to improve water solubility of the corticosteroids, vitamins and benzodiazepins. Phenytoin is another example of a drug where the prodrug approach has been considered to increase water solubility (Stella et al., 1998). A series of prodrugs of phenytoin with improved aqueous solubilities have been evaluated. The disodium salt of the phosphate ester of 3-hydroxymethyl phenytoin was found to be 4500 times more soluble than phenytoin and the parent compound was generated rapidly in in vivo. It should be emphasized, however, that an increase in aqueous solubility does not necessarily result in an improved bioavailability. In order to be able to permeate the lipophilic epithelial cell membranes lining the gastrointestinal tract, a drug molecule must posses lipophilic properties. Thus for highly polar compounds, administration of less polar, more lipophilic prodrugs may improve absorption. Examples are bacampicillin, pivampicillin, and talampicillin, which are prodrugs of highly polar ampicillin.

If chemical modification is not feasible, formulation approaches can be used to improve oral absorption of poorly soluble drugs. Usually the initial approach used to improve solubility of an insoluble drug in water is to form water-soluble salts. The classic review on pharmaceutical salts by Berge et al. (1977) needs a special mention. If salt formation is not possible, e.g. too unstable, or does not render the molecule sufficiently water soluble, a series of formulation approaches may be investigated. Often a useful approach to increase the aqueous solubility of an ionizable drug is pH adjustment. The next approach most frequently tried is the use of water-miscible cosolvents. Other approaches include the use of surface-active agents, complexing agents, liposomes, salting-in, salting-out, clathrate formation. Complexation of water insoluble drugs usually involves the incorporation of the drug within the inner core of the complexing agent so that the outer hydrophilic groups of the complexing agent interacts with water, rendering the drug more soluble. Cyclodextrins are the commonly used complexing agents that form inclusion complexes in both the solution and solid states. For example, the solubility of miconazole in water increased to nine- to 55-fold in the presence of cyclodextrins leading to increased dissolution rate (increased to 28–255-fold) (Tenjarla et al., 1998). In the same study oral bioavailability of miconazole in rats increased to 2.3-fold by complexation with hydroxypropyl b-cyclodextrins and transdermal diffusivity increased to two- to eight-fold (Tenjarla et al., 1998). Solubility of many other poorly soluble drugs such as hydrocortisone, digitoxin, diazepam, indomethacin, iotraconazole, kynostatin, pilocarpine, cinnarizine, naproxen, and thiabendazole has been improved by natural and substituted cyclodextrins (Muller and Brauns,

1985; Putteman et al., 1997; Uekama et al., 1998).

3.2. *Permeability and partition coefficient*

The ability of a molecule to cross the biological membranes (permeability) is a very important biopharmaceutic parameter that governs the absorption, distribution, metabolism and excretion (pharmacokinetics) of a drug.

Enroute to its biophase, the drug has to partition between the lipid biomembranes and the aqueous biological fluids. Although constituents vary from one membrane to the other, major constituents of biomembranes are phospholipids, cholesterol, sphingolipids, and glycolipids. All of these lipids are amphipathic in nature. Therefore, to successfully cross the various biomembranes and to reach its site of action, any drug molecule should have a balance between hydrophilic and lipophilic properties. The octanol–water partition coefficient (*P*) is a physical property used extensively to describe a chemical's lipophilic or hydrophilic properties. It is the ratio of unionized compound in mutually saturated octanol and water. Since, *P* values may range several orders of magnitude, the logarithm (log *P*) is commonly used for convenience. The best choice out of various non-polar and slightly polar solvents available is *n*-octanol (Smith et al., 1975) because, it mimics the biological membranes in several aspects: *n*-octanol has a saturated alkyl chain, it has a hydroxyl group that can act as both hydrogen bond donor as well as acceptor, it dissolves water to the extent of 1.7 M, and its solubility parameter ($d_{octanol}$=10) is close to that of biological membranes, for example skin (d_{skin}=10). This combination of lipophilic chains, hydrophilic groups, ability to take up water molecules and similar solubility parameter gives *n*-octanol properties very close to those of natural membranes. As the physiological pH is 7.4 partition coefficients are calculated using *n*-octanol buffer pH 7.4 system and is more appropriate for drugs ionizable at physiological pH. Partition coefficients that are measured at a given pH are known as distribution coefficients (*D*) and is defined as the ratio of the concentration of compound in the organic phase to the concentration of both ionized and unionized species in the aqueous phase at a given pH (Scherer and Howard, 1977).

Though the log *P* or log *D* values measured for various drugs and xenobiotics using *n*-octanol as non-aqueous solvent correlated well with permeability, distribution and other pharmacokinetic parameters, these values had no correlation with the same pharmacokinetic parameters of peptides (Burton et al., 1992), amide containing drugs
(Smith et al., 1996) and many basic amines (Austin et al., 1995; Barton et al., 1997). Many basic amines show a much higher partition into membranes than one would expect considering their log *D* (octanol/water) values (Barton et al., 1997). This is because *n*-octanol can only support the efficient partitioning of the neutral form of the drug whereas biomembrane, as a consequence of having negatively charged phosphate head groups, can support the partitioning of both neutral and positively charged form of amines. In such cases membrane/water partition values log *D* (membrane−water) and D log *D* [(octanol/water)−(membrane/water)] are good models for permeability. In the case of peptide drugs log *D* (octanol/water) values will not correlate well with permeability data because, peptide drugs have many polar functionalities that form hydrogen bonds with hydroxyl groups in aqueous phase. As octanol can also form hydrogen

bonds with peptides and amide drugs, it will give misleading higher partition values that will not correlate with permeability. However, correlations can be achieved between the permeabilities of peptides and the number of potential hydrogen bonds that peptides can make with water, suggesting that desolvation of the polar bonds in the molecule is a determinant of permeability. Hence, partition coefficients between heptane–ethylene glycol or the differences in partition coefficients between octanol buffer and isooctane/cyclohexane buffer (D log *P*), both of which are experimental estimates of hydrogen bond or desolvation potential are good descriptors for permeability of peptides through intestinal membrane as well as blood– brain barrier (BBB) (Abraham et al., 1994; Chikhale et al., 1994; Von Geldern et al., 1996). Von Geldern and colleagues reported the improvement of oral absorption profile of azolebased ET_A-selective antagonists through rational structural modifications suggested by D log *P* [(octanol/water)−(cyclohexane/water)], which tends to emphasize the hydrogen bonding capacity of molecule, relative to its hydrophobicity. The hydrogen bonding capacity was reduced using a series of urea modifications. The resulting compounds showed a good correlation between D log *P* and small intestinal absorption in rat.

Though there is no general rule that can be applied across the vastly diverse drug molecules, some generalizations can be made within a homologous series of drug molecules. Within a homologous series, drug absorption usually increases as lipophilicity rises and is maintained at a plateau for a few units of log *P* after which there may be a steady decrease, giving a parabolic relation (Navia and Chaturvedi, 1996). Similar parabolic relationship has been found between mainly to the increased affinity of drugs for the enzymes (Martin and Hansch, 1971).

Table 4
List of computer software available to calculate log *P*

Software program	Method	Reference
ragmental approaches		
PROLOGP–cdr	Original Rekker	Rekker and Mannhold, 1992
SYBYL	Revised Rekker	Rekker and Mannhold, 1992
CLOGP	Leo–Hansch	Hansch and Leo, 1979
KLOGP	Klopman	Klopman et al., 1985
KOWWIN	Meylan–Howard	Meylan and Howard, 1995
tom-based approaches		
PROLOGP	Broto	Broto et al., 1984
–atomic PROLOGP –atomic5	Ghose–Crippen	Ghose and Crippen, 1986
MOLCAD	Ghose–Crippen	Ghose and Crippen, 1986

CHEMICALC2	Suzuki	Suzuki and Kudo, 1990
Molecular propert	*y based approaches*	
BLOGP	Bodor	Buchwald and Bodor, 1998
SciLogP	Bodor	Buchwald and Bodor, 1998
ASCLOGP	van de Waterbeemdet al., 1996	Van de Waterbeemd
HINT	Abraham–Kellogg et al., 1991	Kellogg

log *P* and biological activity. Saha et al. (1994), Merino et al. (1995) showed a sigmoidal relationship between absorption rate and log *P* in a series of 6-fluoroquinolones and b-blockers respectively. In general, log *P* values between 0 and 3 constitutes an optimal window for passive drug absorption. A log *P* value below 0 means that the compound is hydrophilic, and hence it will have a good solubility but it may have poor permeability. Whereas, a log *P* value far higher than 3 means that the compound is highly lipophilic, hence, tends to favour absorption, and renders the compounds more susceptible to metabolism and/or biliary clearance (Toon and Rowland, 1983; Humphrey, 1989). The influence of lipophilicity on the metabolic clearance of drugs is attributed If a lead compound has modest activity and has log *P* value below zero, it would be reasonable to synthesize an analogue with a higher log *P*. At this stage predictive methods of log *P* are highly valuable. Although, none of them give accurate values as they did not include all the effects of molecular confirmation, proximity, tautomerism and potential for hydrogen bonding. into the calculation procedure, these methods are still useful and practised commonly in pharmaceutical industry since experimental measurement can be difficult, time-consuming, and/or expensive (Kristl et al., 1999). The literature contains many methods for estimating log *P* and can be mainly categorized into three major groups: the most common fragment constant methods (Fujita et al., 1964; Hansch and Leo, 1979; Rekker and Mannhold, 1992; Meylan and Howard, 1995), methods based on atomic contributions (Broto et al., 1984; Ghose and Crippen, 1986; Suzuki and Kudo, 1990) and those based on molecular properties (Sasaki et al., 1991). To simplify computation calculations of these methods many computer programs are available and are listed in Table 4. The effect of lipophilicity on oral absorption is best exemplified by the classical study of barbiturates (Schanker, 1960), b-blockers (Taylor et al., 1985; Saha et al., 1994), bisphosphonates (Lin, 1996) and 6-fluoroquinolones (Merino et al., 1995). Development of orally bioavailable peptide based renin inhibitor A-72 517 by Kleinert et al. (1992) is a good example for improving oral absorption by altering the physicochemical properties like partition coefficient and solubility. Initially Kleinert and his colleagues developed A-64 662 (enalkiren) a first generation renin inhibitor that is effective intravenously but shown to

lack oral bioavailability (Kleinert et al., 1992). Then they devised A-72 517 a close analogue of A-64 662 to improve oral bioavailability by improving oral absorption as well as metabolic stability. The P_2-site histidine and NH_2-terminal b-alanine residues of A-64 662 are more basic than their counterparts in A-72 517 and contain nitrogen-bound protons capable of forming hydrogen bonds (Fig. 4). Consequently, A-72 517 is the more lipophilic compound with a log *P* of 4.6 (in octanol–water, pH 7.4), as compared with a log *P* of 2.6 for A-64 662, and the aqueous solubilities of the salts are 10 mg/ml and 100 mg/ml, respectively. These physicochemical properties of A-72 517 along with its proteolytic stability made it orally bioavailable (53% in dog). The discovery of fluconazole (Richardson, 1993), a systemic antifungal, is another good example that substantiates the importance of lipophilicity in drug discovery and development. Pfizer's tioconazole was clinically effective against fungal infections of the vagina and skin but failed to act systemically when given intravenously or orally. Biopharmaceutic and pharmacokinetic studies revealed that although this drug was absorbed reasonably well from GI lumen, it was subject to extensive first pass metabolism and high protein binding. Efforts to decrease lipophilicity of the molecule lead to the novel systemic antifungal agent, i.e. fluconazole. This clearly substantiates the fact that the lipophilicity of a drug not only affects its absorption but also its metabolism, protein binding and distribution. Generally, the higher the lipophilicity of a drug, the higher its metabolism, the stronger its binding to protein and greater its distribution (Toon and Rowland, 1983). High protein binding may result in pharmacokinetic drug interactions when concomitantly given with other drugs.

4. Pharmacokinetics in drug development

As pharmacokinetics of a drug molecule dictate its availability at its site of action, they are very important in drug selection and optimization. Over the past decade the application of pharmacokinetic data in drug development has gradually increased. Today it is well recognized that successful drug development programs include supportive pharmacokinetic data. Pharmacokinetics serves as a useful tool in the drug development process both in terms of therapeutics and in defining drug disposition characteristics. In this section critical pharmacokinetic parameters and high throughput in vitro and in vivo screening tools to determine the pharmacokinetic parameters are discussed.

4.1. *Critical pharmacokinetic parameters in drug de6elopment*

Ten critical pharmacokinetic parameters; clearance, effective concentration range, extent of availability, fraction of the available dose excreted unchanged, blood/plasma

concentration ratio, half-life, toxic concentration, extent of protein binding, volume of distribution, and rate of availability should be determined for each new chemical molecule both in test animal and in man (Benet, 1993). Role of pharmacokinetics in various stages of drug development were given in Table 5. All the aforementioned pharmacokinetic parameters critical for drug candidate selection can be explained from the following equations, which hold good at steady state during therapy.

$$FD/\mathrm{t}=C_{ss}\mathrm{CL} \quad (2)$$

$$\mathrm{CL}=0.693V_d/t_{1/2} \quad (3)$$

$V\mathrm{d}=V\mathrm{p}+(V\mathrm{t}\,f\mathrm{up}/f\mathrm{ut})$ (4) where F is extent of bioavailability, D is dose, t is dosing interval, C_{ss} is steady state concentration of drug in plasma, CL is total clearance, V_d is volume of distribution, $t_{1/2}$ is elimination half-life,

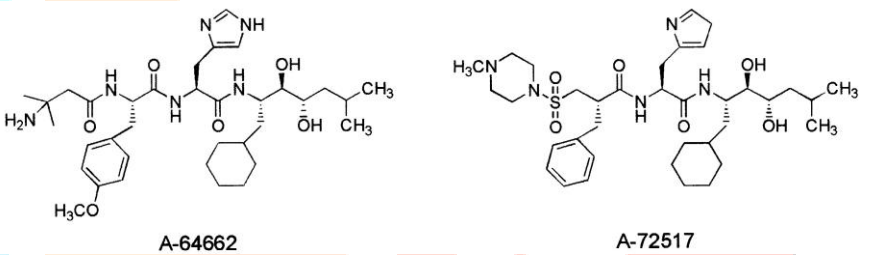

Fig. 4. Chemical structures of A-64 662 and A-72 517.

Table 5
Role of pharmacokinetics in various stages of drug development

Stage of development	Role of pharmacokinetic studies
Selection of drug candidates for development	Consideration of the pharmacokinetic profile desired in connection with known biotransformation processes; explorative in vitro studies.
Preclinical development	Design and interpretation of pharmacological and toxicological investigations also with respect to species differences.
Clinical development: phase 1, 2 and 3	Establishing dosage regimens, absolute/relative bioavailability, identification of metabolites and evaluation of their contribution to the biological profile of the drug. Studies in special patient groups at potential risk (age, disease, metabolic disorders, co-medications) to adjust dose regimens.

V_p is the volume of plasma, V_t is the tissue volume and f_{up} and f_{ut} are the unbound fractions of drug in plasma and tissue respectively.

CL, C_{ss}, and F are necessary to define the appropriate dosing rate (amount/day) of a drug by a particular route of administration (Benet and Williams, 1990). In vitro CL rates determined using human systems (enzyme isoforms, microsomes, hepatocytes) are predictive of the in vivo CL of many lipophilic drugs in man (Smith and van de Waterbeemd, 1999).The in vitro CL rates can be determined from V_{max}/K_M (maximum velocity/Michaelis–Menten constant) determinations for the major metabolic pathway.

Fraction of the available dose excreted unchanged (f_u) and blood/plasma concentration (C_{ss}) are necessary to make one of the most important judgements in drug development process. That is, can this drug be successfully marketed as an oral dosage form in man? (Benet, 1993). Knowledge of f_u allows one to estimate non-renal clearance, which may be assumed to represent hepatic clearance. If the total drug available is excreted unchanged in urine, it implies that no first pass, no dose dependent pharmacokinetics, no enzyme induction and enzyme inhibition by the drug. In such cases, which of course is rare, less attention may be paid to metabolic studies. But, in cases where most of the drug is eliminated in the form of one or more of its metabolites ($f_u$$0), enzymes responsible for metabolism, metabolite profile and pharmacodynamics, toxicokinetics and pharmacokinetics of metabolites must be studied extensively at the early stages of drug development. Such drugs are prone to show high first pass metabolism, dose dependent pharmacokinetics and pharmacokinetic drug interactions. Hence, drugs with f_u close to zero are not suitable for oral drug delivery. By decreasing the lipophilicity of such drug molecules either by addition of polar groups or removal of non-polar groups that are away from pharmacophore, one can make such molecules suitable for oral delivery.

Half-life is an important parameter in therapeutics, since this parameter defines the dosing interval at which drugs should be administered.

Half-life also describes the time required to attain steady state or to decay from steady-state conditions after a change in the dosage regimen. Eq. (3) describes the half-life relationship for a drug that appears to follow one-compartment body kinetics. It is always preferable to select a drug candidate with long elimination half life as it allows less frequent dosing and hence, can improve patient convenience and compliance.

Once the drug is in systemic circulation it will then distribute to all the tissues at a particular rate depending on its physicochemical characteristics such as lipophilicity and charge. To enter various organs and finally to cells the drug has to cross several rate limiting barriers such as cell membranes and some physiological barriers (BBB, blood placental barrier). Hence, lipophilicity is a good descriptor for determination of distribution characteristics such as volume of distribution (V_d). V_d is a hypothetical volume of body fluid that would be required to dissolve the total amount of drug at the same concentration as that found in the blood. V_d can be estimated from partition coefficient (P) and the fraction of protein binding (p) as shown in Eq. (5) in which BW is body weight in grams (Ritschel and Kearns, 1998).

$$V_d=(0.0955P+1.2232)(1-p)BW \text{ (ml)} \quad (5)$$

In general, higher the lipophilicity higher is its distribution. Hence lipophilic drugs have high V_d. Hydrophilic drugs and drugs ionizable at physiological pH will be confined mostly to blood compartment and therefore have low V_d. In case of basic drugs ionized at physiological pH such as quaternary amines, V_d is high due to ion–pair interaction between the positively charged basic centre and negatively charged phosphate head groups of biomembranes. In case of distribution to brain, the general rule, i.e. higher the lipophilicity higher is the distribution, will hold good only within a certain range. Drugs with extremely high lipophilicity cannot permeate easily through BBB. It is believed that P-glycoprotein, located on apical surface of the endothelial cells of the brain capillaries, is responsible for the poor permeability

of highly lipophilic compounds through BBB (Lin and Lu, 1997).

For drugs whose site of action is located intracellularly (such as antisense drugs) V_d should be very high. Otherwise most of the administered dose of the drug will be confined to blood compartment and sufficient amount may not reach the actual site of action. In other words more dose may be required to achieve the sufficient levels of drug at the site of action at which it may show some adverse effects. As V_d is high, i.e. more of the drug is in tissues and hence, less amount of dose administered is available for clearance leading to lower clearance rates. As a result longer elimination half-lives ($t_{1/2}$) can be achieved with high V_d. The relationship between V_d, CL and $t_{1/2}$ is shown in Eq. (3). For drugs having low safety of margin and those required only in circulatory system such as anti-arrythmatics and other cardiovascular drugs, low V_d are preferable in order to prevent unnecessary exposure of the drug to the tissues.

Many drugs are bound to plasma and tissue proteins. The extent of protein binding of a drug is influenced by several factors such as lipophilicity, charge, chemical structure and complementarily of the drug to binding sites. Only the free, non-protein-bound fraction of the drug can diffuse across membranes that restrict distribution of drug between vascular compartment and tissues. And only the free drug can exert a pharmacological action. Hence, drug–protein binding can affect the distribution, clearance and pharmacodynamics of a drug. On an average an adult human has approximately 0.18-kg plasma proteins and 10-kg macromolecular structures such as proteins and nucleic acids in tissue (Ritschel and Kearns, 1998). Considering the apparent volume of distribution of a drug and its relationship to plasma and tissue distribution, the V_d can be characterized as described in Eq. (4), from which it is clear that both plasma and tissue protein binding influence the volume of distribution. But the quantitative relation of plasma to tissue proteins indicates that plasma protein binding should not be of significant influence on the distribution equilibrium of drugs of extremely high lipid solubility unless they have an extremely high affinity for plasma proteins. Plasma protein binding is of significant influence on the distribution equilibrium if the drug is polar.

4.2. *In vitro studies*

With the on-going emphasis to reduce development time, it became imperative to introduce approaches for evaluating absorption and metabolism prior to selection of a development candidate. Advances in in vitro methodologies along with computational and physicochemical approaches for predicting drug absorption are required to meet the increasing demands. Advantages of these in vitro techniques include: rapid evaluation of membrane permeability and metabolism, ability to identify and characterize mechanisms and pathways of drug transport and metabolism, methods for enhancing drug permeability and minimizing drug metabolism, minimization of the use of animal studies that are resource intensive; and the potential to use human rather than animal cells or tissues. A list of in vitro methods used to estimate pharmacokinetic parameters are given in Table 6.

4.3. *High throughput in vivo screening in preclinical drug development*

The difficulties with the identification of compounds that possess the desired pharmacokinetic (CL, V_d, $t_{1/2}$, area under the curve (AUC)) profile eventually became severe enough, with the high productivity of combinatorial chemistry, forcing for a re-evaluation of the process used to conduct these studies. Increased throughput in in vivo pharmacokinetic screening has recently been reported by (a) cassette dosing, i.e. dosing multiple compounds to a single animal at one time, and (b) by pooling samples from singularly administered compounds prior to analysis (Berman et al., 1997; Allen et al., 1998; Cox et al., 1999; Shaffer et al., 1999).

In vivo cassette dosing has been investigated as a way to increase throughput of the pharmacokinetic screening of large numbers of potential drug candidates (Berman et al., 1997;

Shaffer et al., 1999). This technique was applied successfully to speed the progress of an a_{1A}-adrenoceptor antagonist to the clinic (Frick et al., 1998). This method studies the concurrent dosing of multiple compounds to a group of animals in an effort to reduce the number of animals, the total time and the cost required to complete the pharmacokinetic evaluations. A cassette may contain five to 20 potential drug compounds with similar characteristics, as well as a structurally related internal standard with well-characterized pharmacokinetics. The internal standard is used to monitor analytical performance and to screen for potential drug–drug interactions. Animal models will be given an oral gavage or intravenous bolus injection of each cassette. Blood samples are collected at specific time points following cassette dosing. Compounds are extracted from blood samples and analyzed using LC–MS–MS methods specifically developed for the compounds in the cassette. The cassette dosing techniques have been successfully applied to a group of 40 or more chemical entities in cancer drugs and GP IIb/IIIa antagonists (Pang, 1998). The advantages of a cassette dosing are that more compounds can be dosed in a shorter time using fewer animals and that the sample analysis is more efficient. Moreover cassette dosing can be efficiently used to create an in vivo pharmacokinetics database to use in developing structure–pharmacokinetic relationships, which will help in defining molecular changes that do and that do not alter pharmacokinetics (Shaffer et al., 1999). However, cassette dosing does have some disadvantages such as drug–drug interactions that can lead to misleading pharmacokinetic results.

An alternative to these cassette dosing studies that obviates the problem of drug–drug interactions is the use of post-dose pooling of samples. By pooling plasma samples collected at equally spaced time points over a specific period, an average plasma concentration can be determined. Multiplication of this average concentration by the time period gives an estimated AUC, and provides a means of ranking compounds based on early pharmacokinetic evaluation. Analysis of the plasma collected at the last time point can give a more accurate AUC determination and can also give some insight into the oral half-life of each compound. This rapid in vivo pharmacokinetic screening method has been successfully used to provide a rank order of over 200 compounds in a specific therapeutic area (Cox et al., 1999).

5. Empirical rules for the selection of drugcandidates based on biopharmaceutics and pharmacokinetics

Biopharmaceutic and pharmacokinetic data can be used to select, from several thousand candidates, those drugs most likely to be orally bioavailable, safe, and effective. A few empirical rules for such an evaluation of drug candidates are summarized below.

Only drugs with appropriate physical properties for absorption should be used. A certain extent of water-solubility is essential for transport to the absorptive site. On the other hand, optimum lipophilicity is required for passive diffusion through membrane barriers.

	drugs and in understanding the binding site morphology and structure–metabolism relationships.	Parikh, 1997; Tarbit and Berman, 1998
S9 preparation	Prepared by a 9000×*g* centrifugation of liver homogenates. Contains both microsomal and cytosolic enzymes and hence, suitable to evaluate both phase I and phase II reactions. High reproducibility can be achieved but show poor in vivo correlations.	Alvares et al., 1973; Czygan et al., 1973

Table 6 (Continued)

Model	Description	References
Microsomes	Prepared by a 100 000×*g* centrifugation of liver homogenates. They mainly contain fragments of the endoplasmic reticulum and lack cytosolic enzymes and cofactors. Hence, suitable to evaluate phase I metabolic reactions only. In combination with selective inhibitors, microsomes can be used to identify isoenzymes responsible for drug metabolism. High reproducibility can be achieved but show poor in vivo correlations.	Lin, 1996; Carlile et al., 1997; Chiba et al., 1997
Isolated hepatocytes	Can be prepared by any one of various methods that are based upon collagenase perfusion. Though viable only for a few hours, suspended hepatocytes are often very successful for the investigation of pathways of metabolism of novel compounds. However, they are not suitable for the investigation of time dependent metabolism, enzyme induction and the expression of many drug-induced toxicities, as they are short lived. Cultured hepatocytes are good alternatives for suspended hepatocytes to perform longer-term investigations.	Le Bigot et al., 1987; Padgham et al., 1992; Carlile et al., 1997; Lave et al., 1997

Liver slices	Precision cut liver slices can be prepared by tissue slicer or microtome. This model is suitable to evaluate both phase I and phase II metabolic reactions. Cell to cell contact and absence of any enzymatic treatment may be considered as advantages. Poor reproducibility but high in vivo correlation can be achieved.	Dogterom, 1993; Balani et al., 1995; Worboys et al., 1996

associated with solubility and permeability (Lipinsky et al., 1997). The 'rule of five' proposed by Lipinsky and his colleagues states that poor absorption or permeation are more likely when: there are more than five H-bond donors; the molecular weight is over 500; the log *P* is over 5; there are more than ten H-bond acceptors.

Compounds with a hepatic extraction ratio close to unity should be avoided. Such drugs show very high first pass metabolism and therefore tend to show poor and variable bioavailability. Hence, such drugs are not suitable for oral administration, which is considered as the most convenient and safe route. At higher doses saturation of the metabolic activity is likely to occur and consequently, all pharmacokinetic and pharmacodynamic responses may show high variability after oral administration of such drugs.

Compounds that are bound excessively to plasma proteins should be avoided. When the fraction unbound is B10%, variations up to several-fold in C_{ss}, V_d, CL and $t_{1/2}$ can occur when bound drug is competitively displaced by metabolites, other drugs, or endogenous/exogenous compounds from food or disease processes.

Depending on the therapeutic class, volume of distribution of the drug candidate should be optimized.

Compounds with higher half-life values are preferable as they allow less frequent dosing and hence patient convenience and compliance.

Drug molecules should lend themselves to efficient and predictable pathways of elimination (the soft-drug concept). There are only a few processes with a virtually unlimited capacity for elimination. Among these are the metabolic processes of hydrolysis and glucuronidation. Glomerular filtration is also unlimited, but it is not effective with lipophilic compounds as they are subsequently reabsorbed.

6. Conclusions

Aim of drug development is identifying potential drug molecules among the hundreds of lead molecules, and getting a safe and more efficacious drug molecule to the therapeutic arena. Pharmacokinetics and biopharmaceutics play an important role as determinants of in vivo drug action. Development of HIV protease inhibitors (indinavir and ritonavir) GP IIb/IIIa antagonists, and the antifungal agent fluconazole are good examples of successfully incorporating pharmacokinetic and biopharmaceutic information into drug development.

The increased number of pharmacologically active compounds identified during the drug discovery process has created a great demand for rapid screening, selection and development of compounds with favorable human pharmacokinetics and safety credentials. New advances in development processes like high sensitive and highly specific analytical methods (HPLC–MS–MS), caco-2 cell techniques, in vitro metabolic studies, in vivo cassette dosing and other biopharmaceutic approaches will definitely enhance the pace and quality of drug development. A great development may be foreseen in the sphere of the use of computer techniques for evaluation and communication of laboratory data and clinical data on the patient.

In conclusion, the role of biopharmaceutics and pharmacokinetics is an indispensable part in accomplishing the goals of drug development, i.e. getting safe and more efficacious drugs with reduced development time and cost.

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