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Perspective

Property-Based Design: Optimization of Drug Absorption and Pharmacokinetics

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Introduction and Property-Based Design

Drugs may be administered via different routes, the oral route generally being the preferred for reasons of ease and compliance by the patient. Absorption via these routes will take place through different membranes forming a barrier to the uptake. In 1932, Collander and Bärlund observed that there are similarities and differences between membranes.[1] Furthermore these authors recognized that molecular size and lipophilicity are two important properties for membrane uptake. In 1971, hydrogen bonding was associated with permeability.[2] The understanding of the role of these properties has obviously grown considerably since these early days, and this review shows these findings led to today's concepts on optimal physical properties.

As medicinal chemists learned in the past, the hurdle between a compound binding with high affinity to a target and a successful drug on the market can be enormous. Poor absorption and related poor pharmacokinetics is one of the main reasons for attrition in the drug development process. It is now widely recognized that physicochemical, pharmacokinetic, and biopharmaceutical properties need to be addressed early in drug discovery. Several papers have discussed the thesis that drugs have distinct properties differentiating them from other chemicals. Using neural networks[3,4] or a decision tree approach,[5] a compound can be predicted as being "drug-like" with an error rate of ca. 20%. Similarly, in a study on drugs active as central nervous system (CNS) agents and using neural networks based on Bayesian methods, CNS-active drugs could be distinguished from CNS-inactive ones.[6] From an analysis of the key properties of compounds in the World Drug Index (WDI), the now well-accepted rule-of-5 has been derived.[7] It was concluded that compounds are most likely to have poor absorption when the molecular weight (MW) is >500, the calculated octanol/water partition coefficient CLOGP is >5, number of H-bond donors is >5, and the number of H-bond acceptors is >10. Computation of these properties is now available as an ADME (absorption, distribution, metabolism, excretion) screen in commercial software such as Tsar (from Oxford Molecular Ltd.). This rule-of-5 should be seen as a qualitative absorption/permeability predictor,[8] rather than a quantitative predictor.[9] The property distribution in drug-related chemical databases has been studied as another approach to understand "drug-likeness".[10,11] These aforementioned analyses all point to a critical combination of physicochemical and structural properties,[12] which to a large extent can be manipulated by the medicinal chemist. We propose to call this tool in medicinal chemistry property-based design (see Figure 1). Regarding properties, we intend physicochemical as well as pharmaco- and toxicokinetic properties. These have been neglected for a long time by most medicinal chemists, who in many cases only had the quest for strongest receptor binding as the ultimate goal.

This Perspective first covers some aspects of the properties of biological barriers. In different sections we will look into the estimation of drug absorption via important barriers including the gastrointestinal tract,

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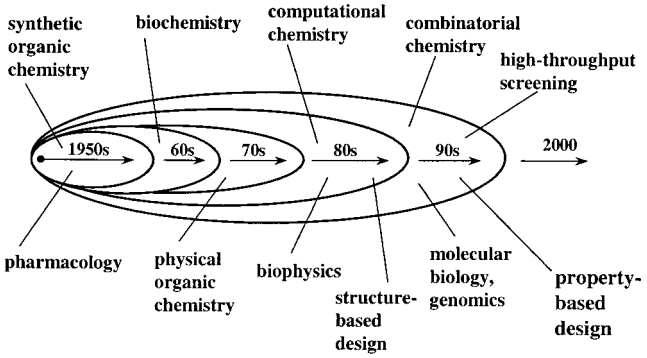


Figure 1. Property-based design, a new tool for the medicinal chemist (modified after ref 13).

blood-brain barrier, and skin. Various approaches for the measurement and computation of physicochemical and molecular properties currently used in the optimization of absorption will be discussed. Finally we discuss the use of physicochemical properties in the design of optimal pharmacokinetic profiles.

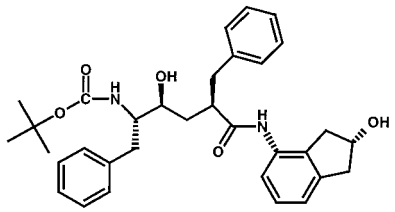
Absorption Barriers

Physicochemical and Biological Barrier. Each cellular membrane can be considered as a combination of a physicochemical and biological barrier to drug transport. Poor physicochemical properties may sometimes be overcome by an active transport mechanism. Before any absorption can take place at all, the first important property to consider is dissolution and solubility. Many cases of solubility-limited absorption have been reported, and therefore solubility is now seen as a property to be addressed at early stages of drug discovery.

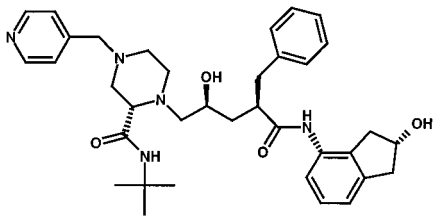
Dissolution. The first requirement for absorption is dissolution of the active compound. Only compound in solution is available for permeation across the gastrointestinal membrane. Solubility has long been recognized as a limiting factor in the absorption process, leading to the implementation of solubility screens in early stages of drug design.[7] Excessive lipophilicity is a common cause of poor solubility and can lead to erratic and incomplete absorption following oral administration. The incorporation of an ionizable center, such as an amine or similar function, into a template can bring a number of benefits including water solubility. A key step in the discovery of the protease inhibitor indinavir (**2**) was the incorporation of a basic amine (and a pyridine) into the backbone of hydroxyethylene transition state mimic compounds such as L-685,434 (**1**) to enhance solubility (and potency).[14]

Dissolution testing has been used as a prognostic tool for oral drug absorption.[15] A biopharmaceutics classification scheme (BCS) has been proposed[16] under which drugs can be categorized into four groups according to their solubility and permeability properties. It has been argued that the principal properties, in fact, are not solubility and permeability but rather the basic components of both, namely molecular size and hydrogen bonding.[17] The BCS has been adopted as a regulatory guidance for bioequivalence studies.

Membrane Transfer. Various mechanisms exist by which substances may be translocated across the membrane of the gastrointestinal tract. These include active



1 (L-685,434)



2 (Indinavir)

carrier-mediated processes; however, these are generally restricted to nutrients and are not normally relevant to the absorption of drug molecules. The two most common for the absorption of drugs are passive transfer by diffusion across the lipid membranes and passive diffusion through the aqueous pores at the tight junctions between cells. These two processes are referred to as transcellular and paracellular absorption, respectively. The ability of a drug to diffuse across the lipid core of the membrane is clearly dependent on physicochemical properties. Thus transcellular absorption is the predominant pathway for lipophilic molecules. In addition, the similarity in membrane structure between species tends to result in similar transcellular absorption across animal species and humans.

The paracellular route of absorption is particularly important in determining the efficiency of absorption of hydrophilic compounds. The restricted diameter of the aqueous pores (typically 3 to 6 Å in humans) means that molecular size also becomes important in the ability of polar molecules to utilize this pathway. Paracellular absorption varies in different regions of the gastrointestinal tract due to varying pore size and frequency. In addition, species differences in absorption have been attributed to variation in pore size, leading to varying efficiency of the paracellular pathway. Most notably it has been speculated that the higher absorption of polar drugs in the dog, compared to rat and human, is due to increased pore size in the dog. Examples of drugs showing this species difference include the β-adrenoceptor antagonists, atenolol and xamoterol. Atenolol (log $D_{7.4} = -1.9$, molecular weight 266) shows complete absorption (~90%) in dog but only about 50% absorption in rat and human.[18−20] Absorption of the larger molecule, xamoterol (log $D_{7.4} = -1.0$, molecular weight = 339), is lower but remains higher in dog (about 36%) than in rat (19%) or human (9%).[21,22] This difference is shown graphically in Figure 2, illustrating the improved model of human absorption of hydrophilic compounds provided by the rat. A study examining the absorption of polar poly(ethylene glycol)s of increasing molecular weight confirmed the higher permeability of dog intestine compared to rat.[23] In this study, absorption in rat decreased sharply from 79% to 2% over the molecular weight range 282 to 591. In contrast, absorption in dog remained 100% up to molecular weight 600, declining to around 13% at molecular weight 900. This was taken

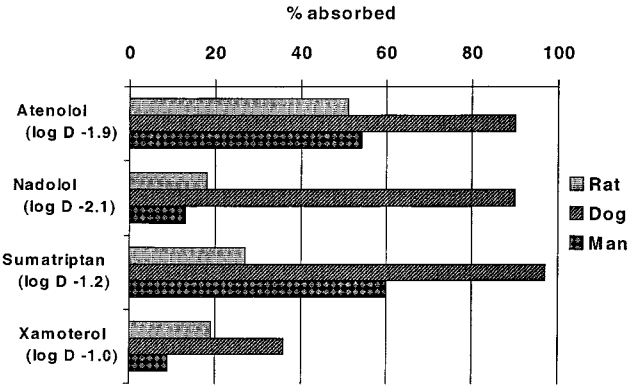


Figure 2. Comparison of extent of absorption in rat, dog, and human for a number of hydrophilic compounds (log $D_{7.4}$ < −0.5), illustrating the greater permeability of dog intestine relative to the other species for polar drugs.

as evidence for larger aqueous pores in dog compared to rat. Thus with regard to animal species modeling absorption in humans, rat appears to be a better predictor than dog.

Human Gastrointestinal (GI) Tract. This organ is well adapted for its role of digesting food and absorbing nutrients. Since most drugs are delivered orally, it is also the major site for drug absorption. Thus, to understand the biochemical processes confronting a drug during its absorption, it is necessary to review the physiology of the GI tract.

There are two principal functions of the gastrointestinal tract epithelium. The first of these is to act as an efficient surface for the absorption of essential dietary elements, such as fluid, nutrients, and electrolytes. The second is to act as a selective barrier between the external environment and the systemic circulation, preventing the entry of xenobiotics which may be potentially harmful to the organs or homeostasis of the body.

The duodenum, jejunum, and ileum (together termed the small intestine) are the major sites of nutrient (and drug) absorption. The human duodenum is approximately 20 cm long, and it is here where the acid of the stomach is neutralized to a more physiological pH. The jejunum and ileum together are about 700 cm long and are responsible for most drug absorption.

The gut wall within the small intestine is particularly well adapted for its role as an absorptive surface with modifications. The internal surface of the small intestine exhibits lumenal folding which increases the surface area for absorption by 3-fold. The gut wall is covered with finger-like projections called villi, which provide a further 10-fold increase in surface area. In addition, the gut wall epithelial cells themselves are polarized such that on the lumenal surface there are millions of microvilli providing for another 20-fold increase in surface area. Thus, overall the surface area available for absorption is some 600-fold higher than would be provided by a simple cylinder. The estimated surface area[24] of the human gut is 200 m^2. With such a large surface area available for absorption, there is the potential for the absorption of molecules that would be harmful to the organism (such as plant toxins in the diet). Consequently, animals have evolved certain protection mechanisms, which are found in the gut wall epithelial cells. It is these protection mechanisms which can limit oral absorption of drug molecules.

The cytochrome P450s (CYPs) are the major enzymes involved in the metabolism of drugs. Most effort has focused on the hepatic CYPs. However, some of the CYP isoforms present in the liver are also expressed in the gut wall epithelium. Thus, it is suggested that CYP2D6 is present in the gut at levels which are 10 to 20% of those in the liver,[25] and therefore its physiological significance is probably rather limited. The major isoform present in the human gut is CYP3A4, which in the small intestine approaches 50% of the hepatic level. The sequence of small intestinal cDNAs for CYP3A4 is identical to that expressed in the liver, suggesting that they are the same protein.[26] Such a study has been performed for the immunosuppressant sirolimus, with pig, rather than human, intestine, where metabolites formed by CYP3A enzyme appeared to be actively transported against a concentration gradient.[27]

P-Glycoprotein (P-gp) was first discovered in 1976, and it was suggested that it played a role in modulating cellular permeability (P stands for permeability).[28] P-Glycoprotein is expressed in vivo in organs including liver, pancreas, kidney, and intestine.[29] In the gut, the expression is highly localized to the apical surfaces of the gut wall epithelium. It is this localization that prompted these authors to suggest that P-glycoprotein has a barrier role, effectively functioning to prevent entry of substances into the animal and reduce the oral absorption of substrate compounds. Further it has been shown that P-glycoprotein mRNA levels increase longitudinally along the intestine, with the lowest levels in the stomach and highest in the colon,[30] an observation that has implications for controlled release technology. Using duodenal mucosal biopsies ($n = 20$), it has been demonstrated that there was a 10-fold variation in the P-glycoprotein mRNA level, suggesting that there will be variability in the expression of P-glycoprotein in the gut leading to potential variability in oral absorption.[31]

In addition to P-gp other transporters may appear to be important in limiting or promoting oral absorption.[32,33] Examples include the oligo-peptide transporter (PEPT1), the monocarboxylate transporter (MCT), and the multidrug resistance-associated proteins (MRP family).

P-Glycoprotein and CYP3A4 May Act Together To Limit Oral Absorption. Many authors have suggested that gut wall CYP3A4 and P-glycoprotein act in a concerted manner to control the absorption of their substrates.[24,27,30,34−36] This is based on the large overlap of substrates between the two[30] and the proximity of their expression within the gut wall. Both these proteins, CYP3A4 and P-gp, share the fact that both have rather indiscriminative binding sites and most likely more than one binding site.

Thus, it is proposed[34] that P-glycoprotein effectively recycles its substrates, allowing CYP3A4 several opportunities to metabolize compounds in the gut. In this way, a small amount of CYP3A4 in the gut wall (relative to the liver content) can exert a profound extraction of the compound.

This certainly appears to be the case for cyclosporin A, which is a substrate for both CYP3A4 and P-glycoprotein, with intestinal metabolism accounting for

up to 50% of oral cyclosporin metabolism. The expression of CYP3A4 and P-glycoprotein in the intestines of 25 kidney transplant patients and their effect on the oral clearance of cyclosporin A have been studied.[36] No correlation between the expression of CYP3A4 in the liver and intestine (i.e., a high expression in liver did not mean a high expression in gut) was found, suggesting that the levels of CYP3A4 in these organs are not coordinately regulated. In addition, there was no correlation between the amount of P-glycoprotein and CYP3A4 in the gut. Interestingly, C_{max} and oral clearance of cyclosporin A could be predicted by comparison of liver CYP3A4 and intestinal P-glycoprotein levels, but intestinal CYP3A4 levels could not be implicated despite a 10-fold variability in enterocyte content. In addition, analysis revealed that 56% of the variability in oral clearance of cyclosporin A was due to liver CYP3A4 and a further 17% due to variability in intestinal P-glycoprotein. Variability in intestinal CYP3A4 did not produce any further variability in oral clearance of cyclosporin A. These authors conclude that the rate-determining step in intestinal extraction of cyclosporin A is cycling due to gut wall P-glycoprotein. If this is the case, cyclosporin A interactions (e.g., with ketoconazole) which were thought to be due to inhibition of gut wall CYP3A4 may actually be due to inhibition of P-glycoprotein. These data are supported by data from others who administered cyclosporin A at various points in the GI tract and showed that absorption was significantly inversely correlated with gut wall P-glycoprotein mRNA levels.[37] In summary, there is a distinct overlap of CYP3A4 and P-glycoprotein substrates. The close proximity of these proteins in the gut wall has led to the suggestion that the two act in concert to complete intestinal first-pass extraction of their substrates. Certainly, this appears to be the case with cyclosporin A.

Design of Drugs for Oral Absorption. To maximize oral absorption, a drug must have sufficient transmembrane flux as well as avoid efflux by P-glycoprotein and metabolism by gut wall CYP3A4. The obvious method to do this would be to design out the characteristics of the molecule which make it a substrate of P-glycoprotein and CYP3A4. Achievement of this goal may well prove to be an insurmountable challenge due to the lack of clear structure−activity relationships (SARs) for P-glycoprotein and CYP3A4.

P-glycoprotein can accommodate a wide range of substrates with the only requirement for transport being a degree of hydrogen bonding in a distinct spatial arrangement and a planar aromatic region.[38] It was concluded that partitioning into the lipid membrane is the rate-limiting step for the interaction of a substrate with P-gp and that dissociation of the P-gp-substrate complex is determined by the number and strength of the hydrogen bonds formed between the substrate and the transporter.[39] Using the multivariate partial least squares (PLS) approach, it was found that properties associated with the size of the molecular surface, polarizability, and hydrogen bonding had the largest impact on the P-glycoprotein-associated ATPase activity.[40]

CYP3A4 may well accommodate a wider substrate range than P-glycoprotein, with SAR suggesting that hydrophobic interactions orientate the substrate within the active site such that sites of electron density are exposed.[41] Thus, modifications designed to increase membrane permeation (i.e., increasing lipophilicity) may well lead to an increased rate of metabolism by this CYP.

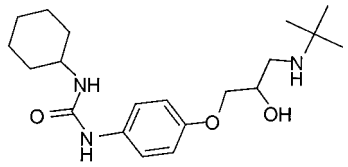
A more pragmatic approach to this problem may well be to concentrate on the factors that give a drug a high permeation rate across the gut wall cell membrane. Thus, we need to balance lipophilicity (taking care to avoid increased metabolism by CYP3A4 in gut and the liver), molecular size, and hydrogen bonding capacity to achieve a high membrane permeation rate. This will lead to a high concentration of the drug within the membrane, effectively saturating the efflux component maintained by P-glycoprotein. In the absence of cycling by P-glycoprotein, the first-pass metabolism by gut wall CYP3A4 will be minimized, leading to a well-absorbed drug. The effect of changing influx, efflux, and metabolism rates on the overall extent of absorption of a compound has been modeled.[42] Crucially, the slower the rate of permeation of the compound across the cell, the longer the residence time in the enterocyte and the more opportunity there is for metabolism of the compound. This means that permeation rate, governed by physicochemistry and P-glycoprotein-mediated efflux, is all important in determining the absorption of a compound. In addition, it is the interplay between efflux and metabolism that allows the gut to exert a greater first-pass extraction than would be expected from its level of metabolic enzymes. Not surprisingly, the model shows that if both efflux and metabolism are inhibited, there is the greatest increase in fraction absorbed.

Overcoming the barriers to the absorption of our drug molecules will be a major challenge in the future as drug leads tend to be of higher molecular weight and often contain more H-bond functionality. We have identified that intestinal CYP3A4 and P-glycoprotein can act as a concerted barrier to drug absorption. Substrates of these proteins are likely to exhibit first-pass extraction in the gut wall, low oral bioavailability, and potentially nonlinear pharmacokinetics. In addition, they will be prone to interactions with other substrates and inhibitors. Overall, a drug discovery aim should be to remove the potential for gut wall first-pass extraction. This may be achieved by tailoring the molecule to exhibit a high permeation rate across membranes by modulating lipophilicity, lowering the molecular size, and reducing the hydrogen bonding of lead compounds, as will be discussed below.

Oral Absorption of P-Glycoprotein Substrates. As transporter science progresses, it is becoming apparent that many marketed drugs are P-glycoprotein substrates. These include protease inhibitors,[43,44] quinidine,[45] fexofenadine,[46] and several β-blockers,[47−49] and for each of these, P-glycoprotein can profoundly affect their disposition.

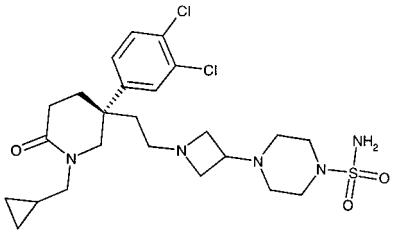
A good example of this is the β-blocker talinolol (**3**). In Caco-2 cells, the transcellular flux of talinolol was highly polarized with significantly greater flux rates in the nonabsorptive (B to A) direction than in the absorptive (A to B) direction.[50] This polarization was concentration-dependent and modulated by the addition of verapamil, indicating that P-glycoprotein was involved. Subsequently, it has been shown that talinolol does

indeed interact with P-glycoprotein[51] and that this has a profound effect on its disposition in the rat.[52] In humans, talinolol exhibits 54% oral bioavailability, mainly due to incomplete oral absorption.[53] It appears that oral absorption of talinolol changes with the site of administration in the gut.[54] There is a progressive decrease in the oral AUC with increasing distance from the teeth, which is consistent with the increase in P-glycoprotein expression in the lower part of the GI tract. In addition, the oral absorption of talinolol is dose-dependent[50] with a 31-fold increase in oral AUC for a 16-fold increase in dose (12.5 mg to 200 mg). This has been ascribed to the saturation of gut wall P-glycoprotein, similar to the effect observed in Caco-2 cells, with a subsequent increase in the fraction absorbed.



3 (Talinolol)

Our laboratories have recently disclosed a further striking example of P-glycoprotein limiting the oral absorption of a drug. UK-224,671 (**4**, a sulfamide NK_2 antagonist) was shown to exhibit 8% oral bioavailability in humans[55] which was reduced to 4% after feeding. Intravenous administration suggested that the hepatic extraction of UK-224,671 was not extensive, indicating that the low oral bioavailability was due in large part to poor absorption from the GI tract. This was confirmed using Caco-2 cell data where the absorptive flux was low ($(1-2) \times 10^{-6}$ cm/s), suggesting that UK-224,671 exhibits low transcellular flux. In addition, the non-absorptive flux was $17-19 \times 10^{-6}$ cm/s, which is indicative of transporter-mediated efflux.[56]

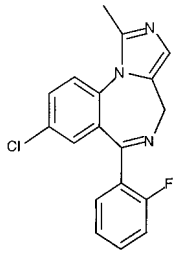


4 (UK-224,671)

The use of P-glycoprotein knockout mice[57] enabled the oral absorption of UK-224,671 to be further examined. The oral bioavailability in wild-type (P-glycoprotein expressing) mice was low (<2%), relating well to the observed human oral bioavailability. However, in mice lacking P-glycoprotein (mdr1a (−/−)), the oral bioavailability was 20%. Thus, UK-224,671 is a P-glycoprotein substrate, and this in combination with its poor membrane permeability leads to the observed poor absorption in both mouse and human.

Thus, many drugs appear to be substrates for P-glycoprotein, and this can have profound effects on their absorption from the GI tract, including limiting the amount of drug absorbed, introduction of variable and site-dependent absorption, and dose-dependent absorption characteristics if P-glycoprotein is saturated. Clearly, if the aim of oral therapy is to provide reproducible and complete absorption, the effect of P-glycoprotein on the drug must be minimized.

Absorption of Cytochrome P450 Substrates. Metabolism of midazolam (**5**), a CYP3A substrate, was examined in mucosa from duodenal, jejunal, and ileal sections of 20 human donors.[58] The CYP3A content was highest in the duodenum and lowest in the ileum. Thus, unlike P-glycoprotein, the concentration of gut wall CYP3A decreases from the proximal to the distal regions. Consequently, the upper small intestine (duodenum) is the major site for intestinal CYP3A-mediated first-pass metabolism, and first-pass metabolism by the gut may be reduced when drug is absorbed at more distal sites in the small intestine.



5 (Midazolam)

The oral bioavailability of midazolam in humans is approximately 36% due to extensive first-pass metabolism.[59] It was shown[58] that in anhepatic patients (undergoing liver transplantation) the gut wall extraction ratio of midazolam is 0.43. This corresponds well with a study in healthy volunteers where estimates of the intestinal and hepatic first-pass extraction ratios were 0.44 and 0.43, respectively.[60] Thus, the gut contributes approximately 50% to the first-pass metabolism of midazolam following oral administration. Also, the variability in the first-pass extraction of midazolam following intraduodenal administration was high (mean value, 0.43; range, 0−0.77), showing that the gut wall can contribute to the significant variability observed with high first-pass extraction drugs.

The gut wall contributes significantly to the first-pass extraction of verapamil (overall oral bioavailability, 16%; gut wall extraction ratio, 0.58; liver extraction ratio, 0.62).[61] A significant gut wall first-pass extraction for cyclosporin A (oral bioavailability, 27%; gut wall extraction, 0.41; hepatic extraction, 0.76) has been shown.[62] In addition, the other immune suppressants, tacrolimus [34,63] and sirolimus,[27] have been shown to undergo significant first-pass extraction by the intestine, as has felodipine[64] and fentanyl.[65] Clearly, the gut wall can play a major role in limiting oral bioavailability for CYP3A substrates.

Assessment of Absorption

Gastrointestinal Tissue Preparations. Tissue preparations for assessing absorption have most commonly been performed using rat tissue. The rat, as previously discussed, providing a relatively good model of absorption in humans. These preparations fall into

two broad categories, either isolated sections of gut tissue or lengths of the gastrointestinal tract perfused in situ. Probably the simplest system is everted rat intestinal sacs, which offer a relatively quick and inexpensive method of measuring drug uptake rates. Everted gut rings are simpler still. However, as the technique measures uptake of drug by the gut tissue, it is generally only practical when radiolabeled drug is available and not necessarily reflective of the absorption process per se. Everted gut sacs offer the flexibility that drug permeation can be investigated in different regions of the gastrointestinal tract, thus providing the opportunity to study regional differences in absorption. This has been demonstrated for the absorption of D-xylose, a passively absorbed monosaccharide. D-Xylose shows 20-fold higher uptake in the small intestine relative to the large intestine using everted gut sacs. This result has also been supported by data using in situ intestinal loop models.[66] Everted gut sacs and rings have been widely used in mechanistic studies of amino acid and peptide transport and have an extensive database in the literature.[67] When considering everted gut sacs as a model of gut absorption, it should be borne in mind that this system measures transport from one side of the gut tissue to the other. In the in vivo situation, transport occurs through the gut epithelium into the blood supply. Hence this is not a true model of the process of absorption. Another drawback to the use of everted gut sacs is the diminishing viability of the tissue over the time course of the experiments. Binding of drugs to tissue is also a concern, as the full permeation potential may not be realized in this isolated system. A refined system for the determination of transport across isolated sections of intestinal tissue is the Ussing chamber.[68] In this preparation, intestinal tissue from any region of the gastrointestinal tract is stripped of underlying muscle and mounted in a chamber as the barrier between two compartments. Both the mucosal and serosal surfaces are bathed with oxygenated buffer solution, and the passage of solute across the tissue is measured by standard analytical techniques. Integrity of the barrier is monitored by measurement of transepithelial electrical resistance across the tissue and permeability of a marker molecule such as mannitol.[69] The Ussing chamber has been used in the assessment of absorption of peptidergic compounds by the paracellular route in the search for orally active GP-IIb/IIIa antagonists.[70] The Ussing chamber technique is also suitable for studying the formation of metabolites formed by intestinal enzymes and their transport across the epithelial barrier. Permeability in the Ussing chamber using rat jejunum has been shown to be highly predictive of permeability in human jejunum and the extent of oral absorption in humans.[71] In this study, the rate of absorption from human jejunum was determined using a single-pass perfusion method. This analysis included a diverse set of passively absorbed drug molecules encompassing molecules absorbed via both the paracellular and transcellular routes (Figure 3) in addition to compounds absorbed by active-carrier-mediated uptake. A similar analysis, for a different diverse compound set, showed a modified Ussing chamber technique to be predictive of both rat and human absorption.[72]

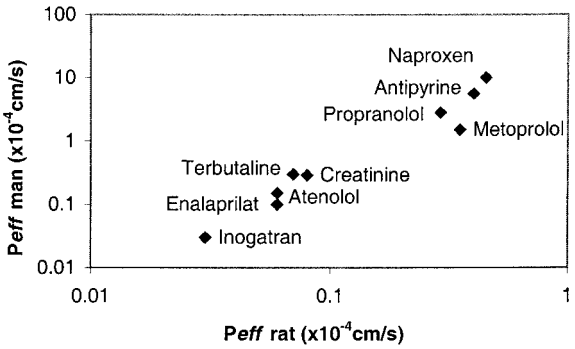


Figure 3. Correlation between permeability in rat jejunum in the Ussing chamber (in vitro) and human jejunum (in vivo) for a range of passively absorbed compounds.[71]

In situ intestinal loop preparations have been widely used for the evaluation of drug absorption over many years (e.g., ref 73). These preparations have been claimed to offer significant advantages over isolated gut preparation methods by virtue of intact nerve and blood supplies in the in situ model.[74] The experimental procedure typically involves isolation of an approximately 20 cm length of rat small intestine, which remains in situ and is perfused in a recirculating manner. Disappearance of test molecules from the perfusate can then be monitored using conventional analytical methods, permitting calculation of an absorption rate normalized for the length of intestine. This procedure has been used to demonstrate the markedly differing permeability of lipophilic and hydrophilic β-adrenoceptor antagonists, practolol and propranolol.[75] A more extensive study was performed comparing the permeability of 11 β-adrenoceptor antagonists ranging in lipophilicity from atenolol (log $D_{7.4}$ −1.9) to penbutolol (log $D_{7.4}$ +2.3) in three different regions of the gastrointestinal tract.[76] This study showed negligible absorption from the stomach for all compounds. Absorption rates were generally similar in the small intestine and colon, with a greater than 10-fold faster rate of absorption observed for the most lipophilic compound (penbutolol) in comparison to the most hydrophilic compounds (atenolol). Within structural series, the model shows excellent correlation with in vivo absorption in both rat[77] and human.[78]

Caco-2 Cell System as a Model for Gastrointestinal Absorption. At present, probably the most widely used system for predicting absorption is by determination of the permeation rate of compounds through monolayers of a human colon adenocarcinoma cell line Caco-2.[79] The use of Caco-2 cell lines in the assessment of gut membrane permeation has expanded rapidly over recent years. In this system, a monolayer of a human intestinal cell line (Caco-2) is grown on a permeability filter support. Currently the 24-well format is used, but a 96-well system has been announced. Measuring the passage of compounds across this cell monolayer from a donor to acceptor compartment then assesses permeability.[80] Good correlations have been observed between permeability across Caco-2 monolayers and extent of absorption.[78] For a series of six nonpeptidic compounds exhibiting between 5 and 100% absorption in humans, measured permeability in Caco-2 experiments ranged from 0.5 to $>50 \times 10^{-4}$ cm/min. All of these compounds

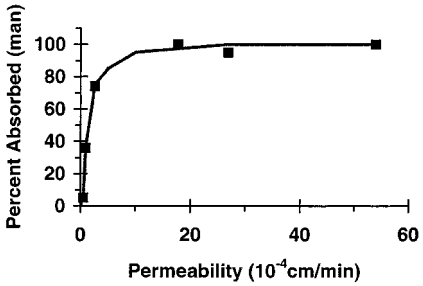


Figure 4. Caco-2 permeability versus the extent of absorption in humans for a series of six compounds.[78]

which showed permeability greater than 20×10^{-4} cm/min were completely absorbed (Figure 4).

While Caco-2 data can provide evidence that a compound will be well absorbed, the steepness of the slope (as shown in Figure 4) means that compounds showing low or modest permeability rates through this monolayer may or may not show a high degree of permeability in vivo. Another complicating factor, which may limit the predictive ability of this system, is the overexpression of P-glycoprotein in this cell line. Caco-2 cell monolayers are polarized with apical and basal surfaces to the cell monolayers. The P-glycoprotein efflux pump is expressed on the apical side of the monolayer and has been shown to mediate the efflux of compounds such as cyclosporin A in Caco-2 experiments.[81] Substrates for this transport protein will be prevented from crossing the monolayer due to efflux back into the donor compartment. P-glycoprotein levels also vary between Caco-2 preparations, and the transporter may also be saturated by some substrates. These variables obviously complicate extrapolation from in vitro to in vivo. Caco-2 cells probably offer the most versatile system currently available for measuring drug permeability. They require considerable time, expense, and effort to establish and maintain the cell line. However, they provide an extremely simple system to measure permeability with straightforward analysis of drug in the buffer solutions that comprise the donor and acceptor media. This system is ideally suited for the measurement of passive transcellular permeation, which is the route of absorption for the majority of drug compounds. In cases where paracellular absorption may be involved, the absence of aqueous pores in the monolayer limits the applicability of Caco-2 cells. Failure to consider the effect of plasma binding can result in an overestimate of basolateral to apical efflux and result in misleading net flux calculations.[82] More realistic sink conditions may be obtained through the presence of human plasma on the basolateral side. Such studies demonstrate that there is scope for further optimization of the Caco-2 model.

Alternatives to the Caco-2 Model. While the Caco-2 system has been extensively used as an absorption model, it is not without drawbacks. One criticism of the model is its already discussed poor representation of the paracellular route. This is due to tight junctions between the cells of the monolayer reflected in high transepithelial resistance (about 400 $\Omega \cdot cm^2$) compared to mammalian intestine (about 60−120 $\Omega \cdot cm^2$). This is considered the main factor in the approximately 50-fold lower permeability of mannitol (a marker of paracellular permeability) in Caco-2 monolayers compared to rat ileum.[83] An alternative colonic cell line, HT29-18-C_1, has shown some advantage in demonstrating reduced transepithelial resistance (100−300$\Omega \cdot cm^2$) and increased permeability to compounds absorbed via the paracellular route.[84]

While the Caco-2 monolayer is more applicable to compound screening than methods using animal tissues, it remains relatively labor intensive with the need for cell culture facilities. The monolayers are also slow to develop to an integral membrane, typically requiring 21 days prior to being suitable for use. An alternate cell line derived from dog kidney is the MDCK cell line, which has the advantage of requiring only 3 days in culture to reach a similar level of membrane integrity to Caco-2 cells.[85] While the MDCK cells are derived from kidney in contrast to Caco-2 cells which are derived from human colon, the two cell lines share many common epithelial cell characteristics. It still is an open debate whether the human colon carcinoma cell line Caco-2 is a better model for human intestinal absorption than the dog kidney cell line MDCK. More detailed characterization of transporters and other cell constituents, as well as differences in permeability related to physicochemistry of the drugs, will be needed. A further cell line of interest is LLC-PK_1, which can be used to express higher levels of CYP3A4 to be able to study the influence of metabolism and transport in a single cell line.[86]

Assessment of the Effect of P-gp on Membrane Transport. Two approaches are currently in use. The first is a direct method consisting of making a comparison between apical to basolateral flux to the reverse process in Caco-2 cells. The second is an indirect method based on measurement of the effect of membrane transport using a P-gp binding assay,[87] an ATPase assay,[88] and an inhibition assay. It can be foreseen that through a rational combination of such screens we will be able to separate passive transport from efflux processes, and thus will be able to study the structure−activity relationships of each of these processes separately.

Measurement and Computation of Molecular Properties

Experimental Approaches. The above-discussed Caco-2 or MDCK cell lines as models for oral absorption estimation have relatively modest throughput. In recent years, the advent of combinatorial chemistry and high-throughput screening has increased the demand for higher throughput in the measurement of physicochemical and pharmacokinetic properties.[89] High-throughput solubility measurements have been developed which can be used in early discovery.[7,90,91] New methods to measure pK_a values are being explored,[92] and an instrument for high-throughput pK_a measurement has been announced.[93] Several approaches for higher throughput lipophilicity measurements have been developed in the pharmaceutical industry,[93] and some are becoming commercially available.[94] A convenient method to measure octanol/water partitioning (log *P*) and distribution (log *D*) is based on potentiometric titration, called the pH method.[95] It gives both the compound's log *P* value and a complete log *D*−pH profile in a single experiment. This method is also suitable for measurement of liposomal membrane−water partition coefficients.[96]

Traditional octanol/water distribution coefficients are still widely used in QSAR (quantitative structure−

activity relationships) and in ADME/PK studies. However, alternatives have been proposed. To cover the variability in biophysical characteristics of different membrane types, a set of four solvents has been suggested, sometimes called the "critical quartet".[97−99] In addition, the 1,2-dichloroethane (DCE)/water system has been promoted as a good alternative to alkane/water due to its far better dissolution properties,[98,99] but it may find little application because of its carcinogenic properties.

Immobilized artificial membranes (IAM) are another means of measuring lipophilic characteristics of drug candidates and other chemicals.[100−104] IAM columns may better mimic membrane interactions than the isotropic octanol/water or other solvent/solvent partitioning system. A further method is called immobilized liposome chromatography (ILC).[105−106] Compounds with the same log *P* were shown to have very different degrees of membrane partitioning on ILC depending on the charge of the compound.[106] The pH-metric method can also be used to study liposome[107] or membrane partitioning. Another relatively new lipophilicity scale is based on micellar electrokinetic chromatography (MEKC).[108]

The two major components of lipophilicity are molecular size and hydrogen bonding.[109] An experimental measure for hydrogen bonding is the difference between the octanol/water and alkane/water partitioning.[110] However, this involves tedious experimental work and it appeared that calculated descriptors for hydrogen bonding can most conveniently be assessed[109] (see below).

The dogma that only neutral species cross a membrane has been challenged.[8] Studies with Caco-2 monolayers also suggested that the ionic species may contribute considerably to overall drug transport.[111] Various ways an ion may cross a membrane have been described.[112] These include transport as ion (trans- and/or paracellular), ion pair, or protein-assisted (using the outer surface of a protein spanning a membrane). Using cyclic voltammetry it was demonstrated that compounds in their ionized form pass into organic phases and might well cross membranes in this ionized form.[113] The difference between the log *P* of a given compound in its neutral form (log P^N) and its fully ionized form (log P^I) has been termed diff(log P^{N-I}), and it contains series specific information and expresses the influence of ionization on the intermolecular forces and intramolecular interactions of a solute.[113−114] It is unclear at present how these latter concepts can be used in drug design. The importance of drug ionization in the in vitro prediction of in vivo absorption was discussed.[115] When the apical pH used in Caco-2 studies was lowered from 7.4 to 6.0, a better correlation was obtained with in vivo data, demonstrating that careful selection of experimental conditions in vitro is crucial to have a reliable model.

Computational Properties. Calculation of many different descriptors is possible using a range of commercially available software packages, such as Sybyl, Cerius2, Tsar, Molconn-Z, Hybot, etc. Due to its key importance, a continued interest is seen to develop good log *P* estimation programs.[116,117] Most log *P* approaches are limited due to a lack of parametrization of certain fragments. For the widely used CLOGP program, a new version avoiding missing fragments has become available.[118] Most log *P* programs are referring to the octanol/water system. On the basis of Rekker's fragmental constant approach, a log *P* calculating for aliphatic hydrocarbon/water partitioning has been reported.[119] These values may offer a better predictor for uptake in the brain. A number of rather comprehensive reviews on lipophilicity estimation have been published.[120−122] Considerable interest is focused on the calculation of hydrogen-bonding capability for use in QSAR studies,[123] for design of combinatorial libraries,[124] and for correlation with absorption and permeability data.[125,126] Several new descriptor sets are based on quantification of 3D molecular surface properties, and these have been explored for the prediction of, e.g., Caco-2 permeability[127] and oral absorption (see below in Computational Approaches to Oral Absorption).

Artificial Membranes and Liposome Partitioning. When screening for absorption by passive membrane permeability, artificial membranes have the advantage of offering a highly reproducible and high-throughput system. Artificial membranes have been compared to Caco-2 cells[128] and, for passive diffusion, found to behave very similar. This has formed the basis of a parallel artificial membrane permeation assay (PAMPA) for rapid prediction of transcellular permeation potential.[129−130] In this system, the permeability through a membrane formed by a mixture of lecithin and an inert organic solvent on a hydrophobic filter support was assessed. While not completely predictive, PAMPA shows definite trends in the ability of molecules to permeate membranes by passive diffusion, which may be valuable in screening large compound libraries. This system is now commercially available.[90] A similar system has been reported based on polycarbonate filters coated with hexane.[131]

Liposomes, which are lipid bilayer vesicles prepared from mixtures of lipids, also provide a useful tool for studying passive permeability of molecules through lipid. This system has, for example, been used to demonstrate the passive nature of the absorption mechanism of monocarboxylic acids.[132] Liposome partitioning of ionizable drugs can be determined by titration and has been correlated with human absorption.[133]

Physicochemical Models for Absorption Processes. Octanol/water partition (log *P*) and distribution (log *D*) coefficients are widely used to make estimates for membrane penetration, including gastrointestinal absorption[134,135] and BBB crossing,[110,136] and correlations to pharmacokinetic properties.[137] Molecular size and hydrogen bonding have been unravelled as the two major components of log *P* or log *D*.[109,138,139] In more recent years it was found that the hydrogen-bonding capacity of a drug solute correlates reasonably well to passive diffusion. Initially Δlog *P*, the difference between octanol/water and alkane/water partitioning, was suggested as a good measure for solute H-bonding,[110,138,140] but more recently it was found that computational approaches to H-bonding are more convenient (see below). Molecular size can be a further limiting factor in oral absorption.[141] The Lipinski rule-of-5 proposes an upper limit of MW 500 as acceptable to complete oral absorption.[7]

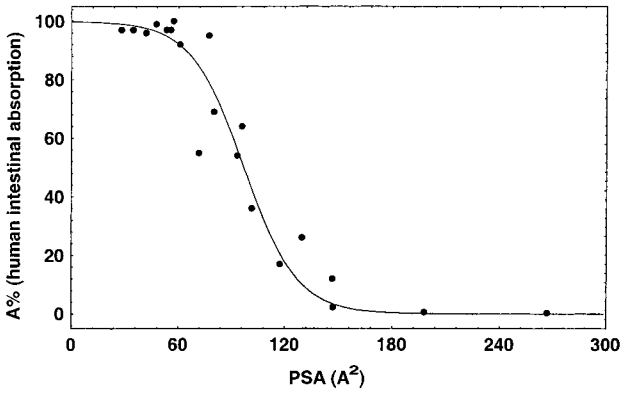


Figure 5. Prediction of oral absorption from the polar surface area (PSA). Sigmoidal relationship between human intestinal absorption and single conformation PSA.[143,144]

Computational Approaches to Oral Absorption. On the basis of the observations that there are optimal ranges for lipophilicity, hydrogen bonding, and molecular size, more advanced computational models could be developed and will be further explored in the future to assist in the design of libraries and further optimization of leads into clinical candidates. Computational approaches are useful in the evaluation of virtual libraries and the prioritization of various options. Computational methods in the prediction of oral drug absorption have been reviewed.[106,142] Both single property correlations using, e.g., polar surface area (see Figure 5)[143,144] as well as multiple variable approaches[106,127,134,145−147] have been successfully used to estimate oral absorption. Most important properties for absorption and permeability appear to be those related to hydrogen-bonding capacity and molecular size of the drug, rather than lipophilicity alone.[106,125] The problem with some of these approaches is that for the medicinal chemist rather obscure or ill-defined descriptors are used such as UNI and PMEP, in MSWHIM,[127] or the integy moment in VolSurf.[146,148] Such descriptors can be difficult to translate back into structural modifications leading to better compounds.

Another approach is based on the so-called solvation equation.[139] In this method, solution-related properties such as solubility,[149] partitioning,[139] blood-brain transport,[150] and gastrointestinal absorption are correlated with a set of five molecular descriptors. These are excess molar refraction, solute polarity/polarizability, solute overall acidity and basicity, and the McGowan characteristic volume. It is important to note that these five descriptors are always forced into the regression equation, despite the fact the some may be intercorrelated,[139] leading in many cases to over-optimistic statistics.

These in silico predictions of absorption are most likely reliable only for compounds that are transported mainly by passive diffusion. More needs to be learned about how to account for transporters involved in active uptake and in secretive efflux, and the role of gut wall metabolism in such predictions (see above in Biological Barriers to Absorption).

Prediction of Oral Bioavailability. Predictive methods for oral absorption are a first step toward the prediction of human oral bioavailability. First attempts in that latter direction have now been published.[151,223] Lipophilicity, expressed as the distribution coefficient at pH 6.5 was found to be a significant factor influencing bioavailability. It appeared also useful to define a new parameter $\Delta\log D$ ($\log D_{6.5} - \log D_{7.4}$) which allows a better classification of the compounds.[151] These and similar concepts will be explored further in coming years.

Estimation of Absorption via Other Absorption Barriers

Blood-Brain Barrier. Molecular factors influencing drug transfer across the blood-brain barrier have been studied by experimental[150,152] and theoretical approaches.[136,153−159] Using dynamic polar surface area (an averaged polar surface area based on a set of low-energy conformations) as a descriptor, a linear relationship was found with brain penetration for 45 drugs,[158] this in contrast to reported sigmoidal curves for oral absorption.[143,144] Brain penetration decreases with increasing polar surface area. Orally active drugs that are transported by the transcellular route should not exceed a polar surface area (PSA) of about 120 Å^2 [136,158] and for good brain penetration should even be tailored to PSA < 100 Å^2 [136] or even smaller <60−70 Å^2.[158] PSA can thus serve as a crude measure to oral absorption and brain uptake, but nevertheless it does not account for active processes involving P-gp, active influx, and metabolism.

Studies on brain penetration of glycine/NMDA receptor antagonists have shown that it is necessary to either keep log P low or pK_a of the acidic compounds high to obtain good CNS activity.[160] In the same study it was shown that only the unbound fraction of drug in the plasma is available to permeate the brain. Besides physicochemical properties as the limiting factor for brain uptake, it is now widely recognized that transporters, particularly P-glycoprotein, play a key protective role to the brain.[161,162] Primary cultures of cerebral capillary endothelial cells have been explored as a model for CNS drug delivery, and it was suggested that studying uptake kinetics in the cells could be more relevant than measuring transmonolayer flux.[163]

Skin. A whole range of experimental skin models is typically used to make estimates of percutaneous absorption. In addition, theoretical percutaneous absorption models have been developed to predict skin permeability.[164] Simultaneous penetration of drug by transcellular and intracellular pathways, as well as movement of drug between these two pathways has been mathematically modeled.[165] Very much similar to the transport through other types of membranes, hydrogen bonding, size, and lipophilicity have been identified as the most important properties for permeation through the human skin.[166−173] The ideal lipophilicity range as measured by octanol/water log P is 2.5 to 6.[166]

Eye. Models of corneal permeability are largely based on similar parameters sets. A fair linear correlation could be established between permeability and $\Delta\log P$ for a series of β-blockers and steroids, and a parabolic relationship was observed with octanol/water partition coefficients.[174] Nevertheless, eye permeation may be more complex as witnessed by specific literature not further discussed here.

Nasal Delivery. The rate and extent of nasal drug absorption may depend on many physicochemical fac-

tors including lipophilicity and molecular weight.[175] Monolayers of human nasal epithelial cells have been used to study the transport and metabolic properties of peptides delivered via the nasal route.[176] Further studies are necessary to establish correlations between in vitro permeabilities in cell cultures and nasal drug absorption in animals and humans.[176]

Optimization of Pharmacokinetics

Role of Pharmacokinetics. It is now clearly recognized that, in the majority of cases, a successful drug candidate requires not only potency and selectivity, but also a suitable pharmacokinetic profile. When traditional drug discovery was largely conducted in animal models, a pharmacokinetic component (albeit in an animal species) was built into the identification of candidate drug molecules. The move toward increased in vitro screening served to highlight the role of pharmacokinetics in in vitro/in vivo discrepancies. Furthermore, retrospective analysis conducted up to 1985 showed inappropriate pharmacokinetics to be the single major reason (39% of all cases) for attrition in drug development programs conducted by the seven U.K.-owned pharmaceutical companies at that time.[177] While adequate pharmacokinetic properties will be essential for drug efficacy, optimal properties provide further opportunity for differentiation from other class members and should be considered the ultimate goal. Indeed, highly favorable pharmacokinetic properties may confer an even greater advantage in ensuring that a drug actually works in clinical practice when the vagaries of patient compliance come into play. This aspect of drug behavior has been termed "forgiveness" and reflects the actual duration of effect after administration.[178] Variability in patient compliance often results in multi-day intervals between doses, hence the ability of a drug to remain efficacious under such regimes becomes highly testing. The long plasma elimination half-life (35 to 50 h) of amlodipine (**18**) provides two or more days of forgiveness. This implicit gain in reliability in use may well have contributed to the market success of this compound despite its relatively late entry into the calcium channel antagonist arena.

The pharmacokinetics of a drug molecule are key in determining the dose required to provide efficacy and the duration for which it remains effective. Both of these facets are influenced by the systemic clearance of the compound.

Clearance as a Determinant of Dose Size. The concentration of a drug at its site of action will determine the level of pharmacological activity. For drugs where the site of action is at the cell surface (e.g., seven-transmembrane spanner antagonists, ion channel modulators), the effective concentration can be regarded as the free drug level in the plasma. Indeed, for many other intracellular targets, the free drug concentration in plasma will be in direct equilibrium throughout the body at steady state. It is generally regarded that, for many receptor antagonists, a clinically significant pharmacological effect requires approximately 75% receptor occupancy.[179] Hence the target efficacious concentration to achieve in plasma can be established based on in vitro potency of a compound (75% receptor occupancy approximates to $3 \times pA_2$ or p*K*i).

For a given dose of compound, the rate of removal from the body will determine the actual "exposure" to the drug. After intravenous administration, this removal parameter is plasma clearance.

$$\text{plasma clearance (Cl)} = \text{dose/area under the curve (AUC = "exposure")}$$

where

$$\text{AUC} = \text{average plasma concentration} \times \text{dose interval}$$

Following an oral dose, the exposure (in terms of AUC) will be governed by the oral clearance of a drug, which will incorporate first-pass extraction by the liver. To take account of free drug levels, oral unbound clearance will determine the actual exposure to unbound drug, i.e., the therapeutically active concentration. Hence, by rearranging the equation above we can see that the dose required to give therapeutic drug levels is determined by the potency of the compound and the oral unbound clearance.

$$\text{dose} = \text{therapeutic concentration} \times \text{dose interval} \times \text{oral unbound clearance}$$

Thus, in order to forecast the dose of a novel oral agent required in the clinical setting, oral unbound clearance is the most important parameter that can be obtained from pharmacokinetic studies.

Clearance and Half-Life. The optimal dose interval for drug administration is often governed by the half-life of a compound. While compounds with wide therapeutic ratios may be more amenable to large variations in peak to trough concentrations in their plasma profiles, in general it is desirable to minimize these in order to prevent adverse effects. In addition, some compounds show a dissociation between pharmacokinetics and pharmacodynamics, hence maintained plasma concentrations are not essential for continued efficacy. However, in the majority of cases, it is desirable for the pharmacokinetic half-life to be sufficiently long to minimize variability in drug concentration and to provide drug levels sufficiently high to drive efficacy. The plasma half-life is in fact a hybrid term that is governed by two pharmacokinetic parameters, plasma clearance and volume of distribution according to the equation:

$$\text{half-life} = 0.693 \times \text{volume of distribution/plasma clearance}$$

Hence half-life can be increased either by increasing the volume of distribution or by reducing the plasma clearance of a compound. The calcium channel blocker, amlodipine, provides an excellent example whereby a very large volume of distribution (>20 L/kg) provides a long plasma elimination half-life (>30 h) despite a moderate plasma clearance (~7 mL/min/kg) in humans.[180] However, while a large volume of distribution can clearly be advantageous, it is often less amenable to manipulation within a drug discovery program, and hence reducing clearance is generally the first approach to extending half-life.

Clearance is therefore a fundamental property of drug molecules in determining the required dose and the

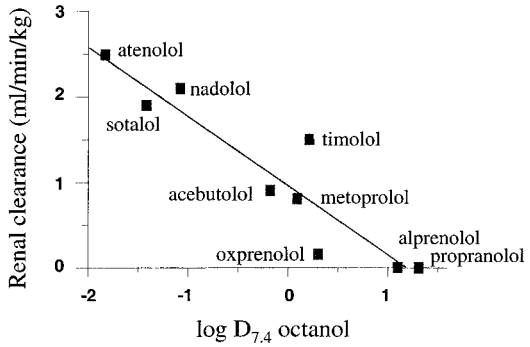


Figure 6. Correlation between lipophilicity (log $D_{7.4}$ values in octanol/water) and renal clearance in healthy human subjects for a series of β-adrenergic antagonists.

frequency of administration, which together may ultimately decide the clinical effectiveness of a new drug treatment.

Renal versus Metabolic Clearance. For the majority of drug molecules, two organs are responsible for clearance, namely the liver and kidney. Lipophilicity is a major determinant of route of clearance due to the dependence of renal clearance on membrane permeability. Renal clearance occurs via filtration of the aqueous component of blood at the kidney glomerulus followed by reabsorption of solutes across the kidney tubule. Compounds that are too polar to undergo reabsorption across this membrane will be eliminated in the urine. Octanol (log $D_{7.4}$) represents a very good mimic of the kidney tubule membrane and thus provides a reliable model of renal excretion. Compounds with log $D_{7.4}$ values above 0 will undergo near complete absorption while compounds with log $D_{7.4}$ values below 0 will be poorly reabsorbed and thus undergo considerable renal clearance. It should be noted that only drug that is not bound to plasma proteins is available to be filtered at the kidney glomerulus, hence plasma protein binding restricts renal clearance. This dependence of renal clearance on log D is exemplified by the series of β-adrenergic antagonists with log $D_{7.4}$ values ranging from about -2 to $+1$ as depicted in Figure 6. In this example, the polar analogues show negligible plasma protein binding while the most lipophilic examples (e.g., propranolol at 93%) show only moderate plasma protein binding. Hence, the unbound renal clearance of propranolol is also low at about 0.14 mL/min/kg.

Hence for hydrophilic compounds (log $D_{7.4} < 0$), renal clearance is an effective means of removal from the body. More lipophilic compounds, on the other hand, will be efficiently reabsorbed in the kidney tubule and will tend to undergo metabolism in order to render them more hydrophilic. Hence the primary role of metabolism is to convert lipophilic molecules capable of crossing biological membranes into polar molecules readily eliminated in the urine. Thus propranolol is mainly eliminated by metabolism[181] with metabolic clearance vastly in excess of renal clearance at about 12 mL/min/kg in humans[182] and accounting for 99% of total clearance.[183]

Pharmacokinetic Scaling from Animals to Humans. To forecast the clinical pharmacokinetic properties of novel compounds, it is necessary to extrapolate from animal or in vitro data to human parameters. Much of the interspecies variation in pharmacokinetic properties can be explained as a consequence of body size. Consequently, it is possible to scale pharmacokinetic parameters to the organism's individual anatomy, biochemistry, and/or physiology in such a manner that differences between species are nullified. Such a process of scaling is referred to as allometry. This approach has been extensively reviewed in its application to the forecasting of pharmacokinetic properties of drug molecules.[184−187] Allometric relationships provide an equation of the general form

$$\text{pharmacokinetic parameter} = A \times \text{BW}^a$$

where A is the coefficient (i.e., the intercept on the y-axis of logarithmically transformed data), BW is the body weight, and a is the power function (slope of the line). This method has been extremely successful for the forecasting of pharmacokinetic parameters that are dependent on blood flow and tissue mass, such as renal clearance and volume of distribution, respectively. Hence for compounds cleared extensively by renal clearance, animal pharmacokinetic data can provide accurate predictions of human pharmacokinetics. An excellent example is the antifungal agent, fluconazole, which is excreted more than 90% as unchanged drug in urine.[188]

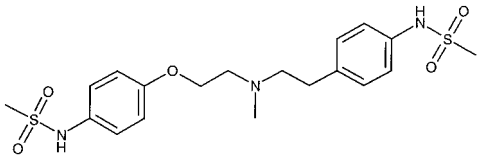
Prediction of Hepatic Metabolic Clearance. The majority of oral drugs are sufficiently lipophilic to enable absorption; this tends to lead to metabolism as the predominant clearance mechanism. Since the liver is the major organ for drug metabolism, a prediction of human hepatic clearance is of great value in candidate drug selection. As already discussed, clearance overall is of prime importance in determining the therapeutic dose size and frequency of a novel compound. Large differences often exist between the metabolic clearance observed in individual species. This limits the utility of a simple allometric approach to the scaling of this parameter to humans. Over recent years the availability of human tissue has increased, and in vitro data generated using this tissue has been shown to accurately predict in vivo clearance. The extrapolation from in vitro to in vivo metabolic clearance in the rat was extensively evaluated using a database of 25 drugs.[189] While hepatocytes were more accurate predictors of high clearance drugs, both hepatocytes and liver microsomes could be scaled satisfactorily. Such extrapolations have also been applied to human metabolic clearance for compounds, including felodipine (**19**)[190] and dofetilide (**6**).[191] The latter example demonstrates the need to know the overall metabolic fate of the molecule in order to scale from in vitro to in vivo intrinsic clearance. Dofetilide (**6**) is metabolized predominantly by N-dealkylation of the tertiary nitrogen, giving rise to three secondary amine products. In the in vitro kinetic studies, only the *N*-desmethyl metabolite was monitored; however, additional in vitro studies were performed to show that this product represented about one-third of the total metabolism in all species. Only when fractional clearance by this pathway was multiplied by 3, to account for the other routes of metabolism, did the in vitro data provide a reliable indicator of in vivo metabolic clearance (Table 1).

The ability of in vitro systems to accurately reflect in vivo metabolic clearance justifies the use of these techniques in early drug discovery programs. However,

Table 1. Scaling in Vitro Metabolism Kinetic Data for Dofetilide to in Vivo Metabolic Clearance[191]

species	V_{max} (pmol/min/mg)	K_M (μM)	intrinsic clearance (mL/min/g)[a]	extrapolated in vivo clearance by N-demethylation (ml/min/kg)[b]	extrapolated in vivo clearance by all pathways (mL/min/kg)[c]	actual in vivo metabolic clearance (mL/min/kg)[d]
male rat	1571	242	6.49	13.16	39.5	58
female rat	358	96	3.73	7.57	22.7	29
dog	127	134	0.95	1.93	5.8	7.2
human	175	657	0.25	0.51	1.5	1.5

[a] Intrinsic clearance = V_{max}/K_M. [b] Using scaling factors.[189] [c] Incorporation of routes other than N-demethylation which was one-third of the total. [d] Reference 192.



6 (Dofetilide)

at the time these studies are conducted, it is unlikely that the overall metabolic fate will be known, hence it is not possible to scale from in vitro to in vivo total intrinsic clearance. Here the objective is to screen for metabolically "stable" compounds within a chemical series in order to incorporate stability into the candidate selection.[193] This will normally be performed by monitoring the disappearance of parent compound without necessarily knowing the structure of the metabolic products. These systems can identify compounds or series of compounds that are resistant to oxidative and/or conjugative metabolism. The choice of system may depend on the chemical series and potential routes of metabolism based on the structure of the molecules. Use of such systems assumes that metabolic clearance will be a limiting factor in systemic exposure. While other factors, such as absorption and plasma protein binding, may clearly play an additional role, minimizing metabolic clearance at an early stage will generally be advantageous. The relatively high throughput of in vitro metabolism systems coupled with advances in analytical technologies in terms of HPLC[194] and mass spectrometric detection[195] allow for rapid assessment at earlier and earlier stages of the drug discovery process. However, it is often prudent to establish an in vitro−in vivo correlation at an early stage in order to support the use of the in vitro system. Analysis using multivariate statistical techniques and artificial neural networks has indicated that human hepatocyte data are a more accurate predictor of in vivo human metabolic clearance than clearance data obtained from animals.[196] However, this analysis was performed on a very limited data set, and a drastically increased number of compounds would be needed to fully evaluate this approach.

One aspect of in vitro metabolism systems, which has yet to be fully assessed, is the impact of nonspecific binding to the tissue preparation. Fundamentally, the intrinsic clearance of the unbound drug in vitro is considered to translate to the intrinsic clearance in vivo.[197] However, when plasma protein binding has been incorporated into clearance extrapolations, it has often yielded a poorer prediction, especially for drugs with high plasma protein binding.[198] It has been suggested that this results from a similar extent of binding in the in vitro preparation, hence underestimating intrinsic clearance in this preparation but canceling out the plasma protein binding in the extrapolation. In the previously mentioned extrapolations for felodipine[190] and dofetilide,[191] tissue binding was shown to be similar to plasma protein binding, and therefore both binding factors could be disregarded in scaling. A study which specifically examined this aspect demonstrated markedly different binding characteristics for basic and acidic drugs.[199] This showed that while serum-free fractions of the basic drugs, propranolol and imipramine, were relatively high (12% and 6%, respectively), their free fractions in microsomes were of a similar magnitude (38% and 16%, respectively). In contrast, the acidic drug warfarin showed a much lower serum-free fraction (0.8%), but minimal microsomal binding (free fraction of 73%). While the in vivo clearance of the basic drugs was relatively well predicted without consideration of the binding in either serum or microsomes, an accurate extrapolation was only obtained for warfarin when binding in both was included. Given the limited number of compounds examined in this study, it cannot be concluded that relative levels of microsomal and plasma/serum binding only need to be considered for acidic drugs, and clearly more data are necessary. Based on the belief that microsomal binding of bases is to the lipid fraction, there is clearly a possibility for vast differences between microsomal and plasma binding to occur. It does, however, indicate that simply screening for metabolic stability may not always provide a direct correlate to in vivo metabolic clearance.

Strategies To Reduce Metabolic Clearance. The strategies to reduce metabolic clearance normally concentrate on a "global" and a functionality level. Reduction of lipophilicity normally accompanies a reduction in metabolic clearance, although there is considerable variation in this correlation. The correlation follows because most drug metabolizing enzymes (such as cytochrome P-450's) have lipophilic binding sites. A more elegant solution is to remove or alter the functionality associated with the rapid rate of metabolism. Four examples are shown in Figure 7. The examples shown focus on the following:

(i) the lability of benzyllic and allylic positions to cytochrome P450 metabolism (particularly CYP3A4) and the use of halogen atoms (particularly fluorine and chlorine) to block oxidation. Metabolic attack of the lead structure included benzylic hydroxylation. This was addressed by substituting oxygen (**8**) for the C-3′ carbon (**7**) to remove this site of metabolism. This step, however, produces an electron-rich phenoxy moiety which is susceptible to para-aromatic hydroxylation. Blocking of the aromatic oxidation with fluorine introduced in the para position was required to produce the eventual more stable substitution.[200]

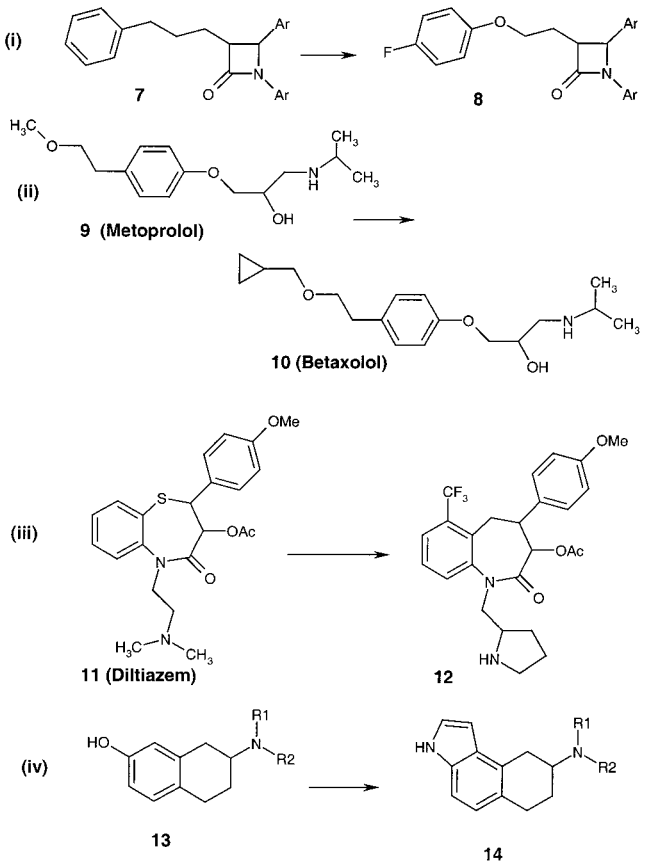


Figure 7. Examples of changes in functionality that attenuate metabolism. The positions of metabolism are marked with an asterisk, and the numbering of the examples relates to the text. Examples i, ii, and iii relate to oxidative metabolism, and example iv relates to conjugation.

(ii) the use of stable functions to create steric factors that do not allow the compound to bind efficiently to the active site of a particular enzyme. Cardioselectivity for β-adrenoceptor agents can be conferred by substitution in the para position of the phenoxy-propanolamine skeleton. The methoxyethyl substituents of metoprolol (**9**) are the major site of metabolism. This reaction is catalyzed by CYP2D6, and the efficiency of the enzyme means that metoprolol shows high clearance. Beside the actual steric bulk of the substituent, which prevents efficient binding to the enzyme, cyclopropyl in betaxolol (**10**) is much more stable to hydrogen abstraction than other alkyl functions and represents an ideal terminal group.[201]

(iii) steric and electronic stabilization of a function to oxidative attack by cytochrome P450. Diltiazem (**11**), a calcium channel blocker, is rapidly metabolized by distinct N-demethylation pathways, by CYP3A4. This was improved through substitution of the benzazepinone ring structure for the benzothiazepinone of diltiazem (**12**).[202] Metabolism studies on this class of compound showed that the principal routes of metabolism were similar to that for diltiazem, and the *N*-desmethyl derivative was equipotent to the parent but much more metabolically stable. This reflects decreased substitution on the nitrogen (2° vs 3°), stabilizing the nitrogen to electron abstraction (decreased radical stability). This stabilization is particularly important, since the electron abstraction is the first step in N-demethylation. The *N*-1 pyrrolidinyl derivatives achieve metabolic stability by the decreased radical stability of secondary compounds to tertiary amines and additionally by steric hindrance afforded by β-substitution.[202]

(iv) use of bioisosteres (such as indole) to replace phenolic functions labile to conjugation processes by glucuronyl transferases. 7-Hydroxy-(amino) tetralin analogues (**13**) are potent agonists of the dopamine receptor. These compounds have very low bioavailability and short duration due to extremely rapid glucuronidation.[203] Substitution of the phenolic hydroxy group with a pyrrolo ring gives a compound series (**14**) with a suitable H-bond donor in the correct position but resistant to glucuronidation.

Volume of Distribution. As previously mentioned, volume of distribution, along with clearance, determines the half-life of a drug molecule (half-life = 0.693 × volume/clearance) and thus will influence the dosing interval. However, as also stated, this parameter is often less amenable to manipulation in a drug discovery program. Part of the reason for this lies in the fact that there is a trend for increasing volume of distribution with increasing lipophilicity.[179] Hence raising the log $D_{7.4}$ to increase the volume of distribution will also tend to increase the susceptibility to metabolic clearance and thus will often be counterproductive. Thus, as metabolic clearance is more dependent on functional groups and substitution patterns, it is often the easier target in a rational drug discovery program.

The presence of charged functions within molecules has a major impact on their volume of distribution. In general, basic compounds have the largest volumes of distribution, and acidic molecules have the smallest. Hence basic compounds which are predominantly present in the tissues will not be available to the organs of elimination (liver and kidney), while a greater proportion of acidic compounds, which are predominantly located in the plasma, will be delivered to these organs. Basic drugs tend to have large volumes of distribution due to favorable interaction with phospholipid membranes. The acidic headgroups of the phospholipid form ion-pair interactions with the basic centers, and thus support the distribution of the ionized drug into the membrane. Acidic drugs, on the other hand, require the unionized drug to partition into the lipophilic membrane, which, given typical pK_a values in the range 3−5 for most organic acids, tends to be unfavorable. In addition, acidic compounds form ionic interactions with the lysine residues on albumin which is present at high concentration in blood. Drug bound to plasma protein is unavailable for distribution, hence limiting tissue penetration of acids. The binding of acidic drugs to albumin is also dependent on lipophilicity, hence lipophilic acids will tend to bind very extensively (e.g., ibuprofen, 99.5% plasma protein bound) and will exhibit volumes of distribution close to plasma or blood volume (0.1 to 0.2 L/kg). Neutral drugs tend to show distribution characteristics between those of acids and bases. Increasing lipophilicity will increase tissue affinity and also affinity for plasma protein, which will restrict movement out of the blood compartment. The overall trend, as with acids and bases, is for volume of distribution to increase with log *D*.

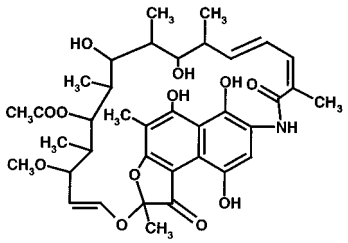
When a prolongation of the pharmacokinetic half-life is a target, the role of the volume of distribution needs

Table 2. Comparison of Pharmacokinetic Properties of of Rifamycin (**15**), Rifampicin (**16**), and Rifabutin (**17**)[a]

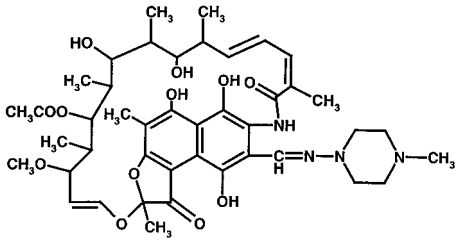
compound	volume of distribution (L/kg)	clearance (mL/min/kg)	half-life (h)
rifamycin	1.2	80	0.33
rifampicin	8.8	32	3.5
rifabutin	266	80	45

[a] Volume of distribution (V_d) and plasma clearance (Clp) are for free unbound drug.

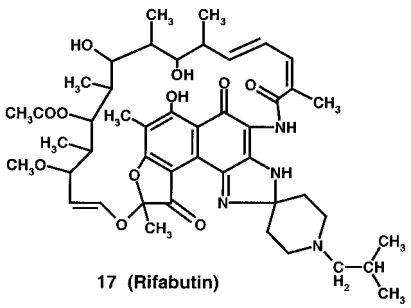
to be considered. If the pharmacological target will accept basic centers within a molecule, then these will generally provide an increase in volume of distribution compared to neutral or basic molecules. This strategy has been followed inadvertently by the medicinal chemist in the search for better solubility. An example of this is the discovery of the series of drugs based on Rifamycin SV [204] (**15**). This compound was one of the first drugs with high activity against *Mycobacterium tuberculosis*. It was not of much utility in the clinical performance due to poor solubility and, hence, dissolution, oral absorption, and a very short duration of action. Many different analogues were produced, including the introduction of basic functions with a goal of increased solubility, until ultimately rifampicin (**16**), a methylpiperazinyl amino methyl derivative, was discovered with much better duration and a successful drug. The basic functionality, however, does not alter clearance but increases volume substantially (Table 2). Duration was enhanced further by the more basic spiropiperidyl analogue, rifabutin (**17**). Again the desirable pharmacokinetic and pharmacodynamic properties are due to effects on volume rather than effects on clearance.



15 (Rifamycin)



16 (Rifampicin)



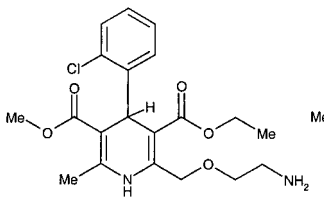
17 (Rifabutin)

Table 3. Comparison of Pharmacokinetic Properties of the Basic Dihydropyridine Calcium Channel Blocker, Amlodipine (**18**), with Neutral Compounds Felodipine (**19**), Nifedipine (**20**), and Nitrendipine (**21**)

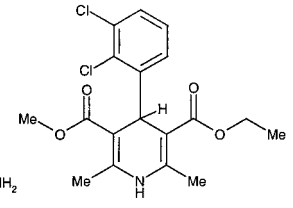
compound	log $D_{7.4}$[a]	volume of distribution (L/kg)	clearance (mL/min/kg)	half-life (h)
amlodipine[205]	1.8	21.4	7.0	33.8
felodipine[206]	4.8	9.7	11.8	10.2
nifedipine[207]	3.2	1.0	8.4	1.9
nitrendipine[208]	4.2	3.8	21	4.0

[a] Medchem database (Daylight Chemical Information Systems, Claremont, CA).

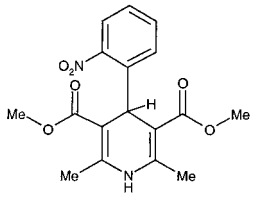
Another striking example is included in the dihydropyridine calcium channel blockers. The basic center of amlodipine (**18**) provides a clear pharmacokinetic advantage over neutral molecules such as felodipine (**19**), nifedipine (**20**), and nitrendipine (**21**). The volume of amlodipine is increased despite an overall reduction in log $D_{7.4}$ (Table 3) in comparison to the other agents. This also serves again as a pharmaceutical advantage in terms of solubility. However, the constraints of pharmacological SAR will often limit the scope of such modifications, hence clearance remains the first line of pharmacokinetic manipulation in most drug discovery programs.



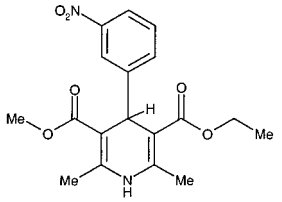
18 (Amlodipine)



19 (Felodipine)



20 (Nifedipine)



21 (Nitrendipine)

Plasma Protein Binding Considerations. Appreciation of free drug exposure is key to understanding the potential efficacy of novel compounds. However, plasma protein binding alone cannot be regarded as either a positive or a negative aspect of a compound. Only when placed into context with pharmacokinetic parameters can valuable insight into the disposition of the molecule be obtained. While it may initially appear that minimal protein binding will increase the efficacious concentration of drug, it will also increase the effective concentration available to the organs of elimination. For example, examine the hypothetical situation where the only property changed within a molecule is the plasma protein binding from 90% to 99% (i.e., free fractions of 0.1 and 0.01, respectively). If this compound had a plasma clearance of 10 mL/min/kg due to hepatic metabolism, the intrinsic unbound clearance would in fact be 100 mL/min/kg. The effect of increased plasma protein binding would not change the intrinsic ability of the liver to clear the compound, and this would remain at 100 mL/min/kg, despite plasma clearance

reducing to 1 mL/min/kg. The overall exposure to unbound drug would also remain constant (AUC_u = dose/Cl_{iu}), for a given dose. The reduction in plasma clearance may initially appear to offer a longer elimination half-life ($t_{1/2} = 0.693V_d/Cl$). However, as only free drug is available for distribution, the total volume of distribution (V_d) will also be reduced by a factor of 10, resulting in the same elimination half-life (in fact if volume of distribution of unbound drug was considered, this, like unbound clearance, remains unchanged). Hence the overall impact of changing plasma protein binding alone is neutral. An exception to this occurs for compounds such as carboxylic acids where the volume of distribution is already limited to the plasma or blood volume (0.1 to 0.2 L/kg). As volume of distribution is already minimal, an increase in plasma protein binding can reduce total drug clearance but leave the volume unchanged, hence extending the half-life. Again, however, the overall exposure to free drug remains the same (AUC_u = dose/Cl_{iu}), although the shape of the plasma concentration time curve will be altered.

It is important to consider differences in plasma protein binding that may occur between species in order to relate pharmacokinetic parameters to safety and efficacy. This is highlighted by the example of zamifenacin, a lipophilic and basic M3 muscarinic antagonist.[209] On the basis of total drug concentration, oral clearance of this compound in humans (0.6 mL/min/kg) was substantially lower than equivalent values in rat (61 mL/min/kg) and dog (26 mL/min/kg). This resulted in markedly higher total drug concentration with dose normalized C_{max} in human 74 and 40 times rat and dog, respectively. However, the free fraction of drug in human plasma (0.01%) was markedly lower than dog (0.1%) and rat (0.2%). Hence free C_{max} values in human were only 8 and 4-fold higher than rat and dog, respectively, on a similar dose normalized basis. In addition, the correlation between pharmacological response in animals and humans was consistent with free drug exposure. Without knowledge of the variation in plasma protein binding across the species, such rationalization of the pharmacodynamics of zamifenacin would not have been possible.

Prodrug Design. A number of successful drugs are on the market in which oral absorption and pharmacokinetics have been improved through the design of prodrugs.[210] A related approach is called soft drug design, where the goal is to integrate metabolism considerations into the drug design process.[211] Important criteria of good products include, among others, stability in the gut and rapid biotransformation to the active parent. On the basis of recent progress and understanding of the role of physicochemical properties, it can be expected that these will be used more in prodrug design than in the past. An example of such an approach is the study of 5-fluoruracil prodrugs in Caco-2 cells.[212]

Prediction of Metabolism

Compounds with either too high or too low clearance, depending on a project's objective, need to be tailored by medicinal chemistry. Therefore, the development of predictive methods for biotransformation is getting increasing attention, so that this input ultimately can be used even at the stage of library design to prevent a flawed template from impacting a whole series. Different techniques are being explored, including QSAR[213] and neural networks, pharmacophore models,[214,215] and protein models.[215,216] Some of these approaches may be combined in an expert system, such as Meteor, Meta, and Metabolexpert.

Examples of the influence of physicochemical properties including lipophilicity on metabolism,[217−219] particularly associated with N-oxidation[220] and glucuronidation,[221] have been discussed. These relationships generally are merely trends rather than real statistically relevant and widely applicable correlations.[219] Another approach consists of the assessment of reaction energetics to predict cytochrome P450-mediated reactions.[222] It is expected that in the future a further increase will be seen in applications of predictive metabolism based on a combination of approaches.

Future Directions

High-throughput screening and combinatorial chemistry have made it possible to increase the chances to find a hit within a drug discovery program. Such a hit can then be further developed into clinical candidates using traditional medicinal chemistry or again some automated process using parallel synthesis. Nevertheless, attrition of the eventual development candidates is still very high, mainly due to toxicity and/or poor PK properties.

Drug design has now come to a stage where an integrated approach including structure-based design and property-based design become the basic concept in the pharmaceutical industry. One expression of this is that, in most companies, departments of Drug Metabolism and Pharmacokinetics have moved from Development to Discovery. Another sign is the fact that most companies now have facilities to measure a number of properties identified as the sources of attrition when these have not previously been addressed in an early phase. Key among these properties are solubility, permeability, absorption, chemical and metabolic stability, and toxicity. In recent years, insight has grown that many of these properties are in some way related to molecular size and hydrogen bonding of the compounds, two simple and appealing properties that the medicinal chemist can manipulate. A challenge will be to define the most appropriate descriptors for both molecular size (e.g., molecular weight versus molar volume) and hydrogen bonding. This appropriateness may well depend on the property one may wish to estimate. Lipophilicity, often expressed by the octanol/water distribution coefficient, is also a combination of these two fundamental physicochemical properties. Various attempts have been made to use other lipophilicity scales, but none have as yet made a breakthrough in the drug design process. Despite the lack of agreed utility, some parameters may have merits for the description of particular distribution phenomena, e.g., the use of $\Delta\log D$ for the prediction of CNS penetration.[110]

On the basis of the findings that simple physicochemical properties correlate with oral absorption, a number of in silico models of varying complexity have been developed to estimate gastrointestinal absorption and brain uptake. Though appealing because of their high-

throughput nature, such methods are often limited to compounds with simple permeation behavior. Today's approaches do not yet account for the effects of transporter proteins involved in active uptake and efflux mechanisms.

In vitro metabolism systems are now routinely employed in the drug discovery phase, and the use of artificial neural networks has shown the potential for these studies to reliably predict human metabolic clearance, albeit in a limited number of examples. Good correlation has been demonstrated between in vitro and in vivo clearance modulated by cytochrome P450 metabolism, especially within chemical series. However, reliable prediction of in vivo clearance modulated by other enzyme systems has been less clearly demonstrated. In addition, routine in vitro tools are not yet available to reliably predict tissue distribution and carrier-mediated tissue uptake and excretion—important contributors to the overall pharmacokinetic profile of a novel compound. Until such tools are available, there remains a need for relatively low-throughput animal studies to extrapolate the likely clinical pharmacokinetic profile. Increasingly, our understanding of the underlying physiological processes contributing to this overall profile allow these in vivo studies to be augmented with appropriate in vitro studies and/or physicochemical measurements to identify optimal compounds. Further growth in this knowledge can only increase the use of in vitro techniques in the future.

Despite this progress, the estimation of human absorption and appropriate dose is often problematic. We are only just seeing the first attempts to predict bioavailability from molecular structure. Larger databases of both oral absorption and bioavailability data are needed to underpin such models. Since bioavailability is the result of several additive complex processes, it is unclear currently which mathematical model(s) is (are) most suited for its prediction. Estimations by their nature will always contain errors; the question for the future will be how small we can make these. We expect that further understanding of the role of various transporters in gastrointestinal absorption and hepatic uptake processes will eventually contribute to better PK predictions.

Conclusions

A rapid increase in the understanding of membrane permeation and absorption processes has recently been seen. The finding that permeability can be related to various physicochemical properties has led to the concept of property-based design. The well-known rule-of-5 is a quantitative representation of this concept. Physicochemical properties can be used in the optimization of pharmacokinetics. It is also recognized that much needs to be learned about transporters influencing either active uptake or efflux of orally administered drugs. In addition, it will be important to understand the role of gut wall metabolism and to develop screens to assess its extent. We expect further developments in the field of computational ADME studies (e-ADME) in the years to come.

Biographies

Han van de Waterbeemd studied physical organic chemistry at the Technical University of Eindhoven, The Netherlands, and received a Ph.D. in medicinal chemistry at the University of Leiden, The Netherlands. After doing postdoctoral work at the School of Pharmacy of the University of Lausanne, Switzerland, he held a faculty position at the same institution. He taught medicinal chemistry to pharmacy students at the Universities of Berne and Basel in Switzerland from 1987 to 1997. In 1988, he joined F. Hoffmann-La Roche Ltd. in Basel and moved to Pfizer Central Research UK in 1997. He is director in the Department of Drug Metabolism. His current research interests include studies of molecular properties and their role in drug disposition. Han is secretary of The QSAR and Modelling Society.

Dennis A. Smith has worked in the pharmaceutical industry for the past 25 years since gaining his Ph.D from the University of Manchester following research on the metabolism of diacetylmorphine. For the last 13 years he has been at Pfizer Global Research and Development, Sandwich, where he is Executive Director of the Drug Metabolism Department. Prior to this he was Head of the Metabolism and Pharmacokinetics Unit at Fisons Pharmaceuticals, Loughborough. Initially his interest centered on the metabolism of foreign compounds; however, this soon extended to the rapidly growing area of pharmacokinetics. For the past decade he has been active in promoting the combined use of both pharmacokinetic and metabolism data to help understand the relationship between a compound's disposition in animal species and that in humans. His interests also include the underlying structural and physicochemical characteristics of compounds, which determine their disposition. He has been a committee member of various drug metabolism groups including ISSX, DMDG, and PK/UK and is on the editorial board of *Xenobiotica*. He is active in a teaching role, holding appointments as Visiting Professor at the University of Liverpool and Honorary Senior Lecturer at the University of Aberdeen. His work in training clinical pharmacologists has been recognized by an honorary membership of the British Association of Pharmaceutical Physicians.

Kevin Beaumont studied biochemistry at London University. In 1983, he joined Pfizer Central Research in the Department of Drug Metabolism where he is now Manager. Throughout his career at Pfizer, he has been involved in the study of disposition of novel compounds from first chemical synthesis through late stage development. His current interests include the effects of excretory transporters, particularly P-glycoprotein, on the disposition of discovery compounds.

Don K. Walker studied biochemistry and chemistry at London University. Between 1981 and 1984, he was involved in research into the detection and characterization of inborn errors of metabolism at University College Hospital, London. In 1986, he joined Pfizer Central Research UK. His current role as Manager and Head of Pre-Clinical Development in the Department of Drug Metabolism includes all aspects of drug metabolism from early discovery to post-marketing. He has been a committee member of the UK Drug Metabolism Discussion Group since 1996.

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