CHAPTER 10

IN SILICO APPROACHES FOR PREDICTING ADME PROPERTIES

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Abstract: A drug requires a suitable pharmacokinetic profile to be efficacious in vivo in humans. The relevant pharmacokinetic properties include the absorption, distribution, metabolism, and excretion (ADME) profile of the drug. This chapter provides an overview of the definition and meaning of key ADME properties, recent models developed to predict these properties, and a guide as to how to select the most appropriate model(s) for a given query. Many tools using the state-of-the-art in silico methodology are now available to users, and it is anticipated that the continual evolution of these tools will provide greater ability to predict ADME properties in the future. However, caution must be exercised in applying these tools as data are generally available only for "successful" drugs, i.e., those that reach the marketplace, and little supplementary information, such as that for drugs that have a poor pharmacokinetic profile, is available. The possibilities of using these methods and possible integration into toxicity prediction are explored.

Keywords: ADME, In silico methods, Biokinetics

10.1. INTRODUCTION

One of the recent success stories in modern drug development has been the incorporation of in silico methods for the prediction of ADME properties into the design process (see Chapter 9). Significant savings have been made in time, cost, and animal use because rapid identification and rejection of pharmacokinetically unsuitable drug candidates means non-viable leads are not progressed from an early stage. In the period between 1991 and 2000, late stage candidate attrition due to pharmacokinetic reasons was shown to be reduced by approximately 30% [1]. There is also a growing interest in the application of in silico prediction of ADME properties to the area of toxicology, to improve accuracy in predicting adverse effects of a wide range of compounds (Chapter 11). The rationale for this is clear when one considers how any xenobiotic produces an effect within an organism as described below.

In order for a compound to be able to elicit a biological effect, be that a required therapeutic effect or an unwanted toxic effect, there are two key determinants:

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(i) the intrinsic activity of the compound and (ii) its potential to reach the site of action in sufficient concentration for the requisite time period. Predicting intrinsic activity of compounds in drug design and toxicology is dealt with elsewhere in this volume (Chapters 4, 5, and 11). This current chapter is devoted to the consideration of the factors which determine whether or not a compound is likely to reach a specific site of action and how long it is likely to persist at that specific site and in the body as a whole.

The terminology used to describe these processes can be context dependent as highlighted by d'Yvoire et al. [2]; therefore a preliminary definition of the terms as used in this chapter is provided. Historically the absorption, distribution, metabolism, and excretion (ADME) properties of compounds were studied in relation to drug development. Hence, in general where the study of the movement (i.e., kinetics) of a compound within the body relates to a desirable effect of a therapeutic substance it is generally referred to as a pharmacokinetic (PK) property. As this appeared to restrict the definition to drugs (at therapeutic doses), the movement of toxic substances responsible for deleterious effects is now generally termed toxicokinetics (TK). The distinction between the terms pharmacokinetics and toxicokinetics, however, is not absolute. For example, where a drug produces a therapeutic effect this would be termed as pharmacokinetics; however, where the same drug produces side-effects or an excess therapeutic effect the drug's movement may be referred to as toxicokinetics. This has led to the general term biokinetics (BK), being proposed as a more inclusive term [2]. However, irrespective of terminology, in PK, TK, or BK studies, the fundamental properties of any compound of interest, i.e., its absorption, distribution, metabolism, and excretion (ADME) properties, are the same. Herein the term ADME will be used in discussion of the movement of all xenobiotics within the body.

ADME properties are arguably the most important consideration in determining the true potential of any compound to elicit a biological effect, desirable or undesirable, within the body. In this chapter, an overview of the definition and meaning of key ADME properties, recent models developed to predict these properties, and a guide as to how to select the most appropriate model(s) for a given query are presented.

10.1.1. Overview of Key ADME Properties

The first requirement for the interaction of a xenobiotic with an organism is the uptake of the compound into the body (except for direct acting agents such as topical irritants). Absorption processes govern the transfer of compounds from the external to the internal environment. Uptake is dependent upon the route of exposure to the compound; there are numerous ways in which xenobiotics may enter the body. Absorption occurs across the gastrointestinal tract for food additives, for toxicants persisting in the food chain or water supply and for compounds leaching from food packaging. Absorption via the lungs or nasal mucosa is important for environmental contaminants present in the general atmosphere or in certain work places. Potential dermal absorption must be considered for cosmetics, personal care products, hair

and clothing dyes in addition to general environmental contaminants. In vitro skin permeability measurements can be used to determine uptake across the dermal barrier. In therapeutics, drug formulation science has exploited every potential route of administering a compound into the body.

The percentage of available compound that is absorbed (% Abs) provides a preliminary measure of internal exposure from any route. For oral ingestion a common measure is termed the percentage human intestinal absorption (% HIA). However, absorption alone does not determine systemic availability. Acting in opposition to absorption processes are local, or first-pass, metabolism and active efflux processes (discussed below). Hence bioavailability (F) is often considered a more useful term as this refers to the percentage of available compound that appears in the systemic circulation. F is directly proportional to absorption and inversely proportional to local, or first pass, metabolism and active efflux.

Once a compound has successfully entered the systemic circulation, the next stage is distribution to other sites within the body. Distribution is a crucial consideration as drugs need to reach their intended site of action, but ideally should not distribute to where they may cause adverse side-effects. Toxicants may cause more severe effects in certain organs (such as the brain) than in others. Distribution and uptake into storage sites, such as adipose, has a significant impact on the time for which xenobiotics persist in the body. Distribution generally occurs via the blood stream, although the lymphatic system is relevant for some compounds. Overall the tendency of a compound to move out of the blood and into tissues is given by the apparent volume of distribution (V_d). This is a hypothetical volume into which a compound distributes and is determined from Eq. (10-1):

$$V_d = \text{dose}/C_0. \tag{10-1}$$

where C_0 is the initial concentration measured in blood.

If a compound has a high tendency to move out of blood, the resulting concentration in blood is low and V_d is very large. Conversely, if it has a tendency to remain in blood C_0 will be high and V_d low. The tendency for a compound to remain in blood or move to other compartments is governed by its ability to pass through membranes and the relative affinity for tissue and blood proteins; hence the percentage of plasma protein binding or fraction bound (% PPB or f_b) and tissue binding are key determinants in distribution. As it is free (or unbound) drug that binds to targets the unbound volume of distribution (V_{du}) is often considered, this is shown in Eq. (10-2):

$$V_{du} = V_d/f_u \tag{10-2}$$

where f_u is the fraction unbound (i.e. $1 - f_b$).

The more widely a compound is distributed throughout the body the more sites are available to elicit potentially toxic effects and the longer the compound will persist in the body. Individual tissue compositions can result in differing affinity

for xenobiotics. Tissue:blood partition coefficients (PCs) indicate the likelihood of a compound being taken up by a specific tissue. One of the most important tissue: blood PCs is that for the blood–brain barrier (BBB) as the central nervous system is associated with some of the most significant toxic effects. The ability of a compound to cross the placenta is also of great concern. The properties used to indicate transfer across the placenta are the placental transfer index (TI) or the clearance index (CI).

Uptake into tissues is also influenced by influx and efflux transporters, which have been identified in many tissues. Uptake transporters include organic anion transporting polypeptides (OATPs), organic cation transporters (OCTs), organic anion transporters (OATs), and the organic cation/carnitine transporters (OCTNs).

Efflux transporters act in opposition to uptake processes. In particular, their presence in the gastrointestinal tract, blood–brain barrier and the placenta provides an important protective effect. ATP binding cassette (ABC) proteins have received much attention because of their recognized role in multi-drug resistance, i.e., their expression in tumor cells, resulting in active efflux of therapeutic agents, is known to be responsible for resistance to drug treatment. Examples of these include P-glycoprotein (P-gp), multi-drug resistance-associated protein (MRP2), and breast cancer resistance protein (BCRP). Phase 0 disposition refers to the ability of a xenobiotic to enter a cell where it may elicit a response. Consequently, the presence of influx and efflux transporters modulates biological response by increasing or decreasing cellular entry [3]. The role of transporters is linked with that of the metabolic processes.

Metabolism is the process by which the body converts xenobiotics usually into a less toxic, more polar form that can be readily excreted. However, in some cases metabolism may be necessary to convert an inactive drug into its active form (i.e., for pro-drugs) or it may lead to the formation of toxic metabolites. Phase I metabolism involves functionalization reactions where a polar group is added to, or exposed on, the molecule. These include reactions such as oxidation of nitrogen or sulfur groups, aliphatic or aromatic hydroxylation, de-amination and de-alkylation. The cytochrome (CYP) P450 superfamily of enzymes is responsible for the catalysis of many of these reactions. The isoforms CYP3A4, CYP2D6, and CYP2C9 are responsible for the metabolism of the vast majority of drugs. Whilst CYP1A1/2, CYP2A6, CYP2B1, and CYP2E1 play little role in drug metabolism, they do catalyze the activation of certain pro-carcinogenic environmental pollutants into their carcinogenic form and are therefore of toxicological importance [4]. Phase II reactions may be consecutive to, or independent of, phase I reactions and include the conjugation of a polar moiety to the compound (e.g., glucuronidation, sulfation, or acetylation) enabling renal or biliary excretion of the polar metabolite. Metabolizing enzymes have been found in all tissues of the body but are predominant in the liver, kidney, and intestine. First-pass metabolism is the process by which compounds that are absorbed into the gut travel via the hepatic portal vein to the liver and are metabolized before they reach the systemic circulation. Enterohepatic recycling, whereby compounds are excreted into bile and hence are returned to the gastrointestinal tract for reabsorption may lead to reappearance of xenobiotics in the blood stream

and prolongation of effect. Gibbs et al. [5] discuss the role of skin metabolism in modulating activity of compounds presented to the body via the dermal route.

In terms of metabolism, there are three specific factors of importance:

- the nature of the metabolite (i.e., is it more or less active than the parent?);
- the extent to which it is formed (i.e., does it represent a major or minor metabolite?); and
- the rate at which it is formed (i.e., how much will be present in the body over time?).

Slow rates of metabolism can be associated with persistence and bioaccumulation of xenobiotics within the body, potentially leading to prolonged activity or toxicity.

Phase III disposition refers to the exit of metabolites from cells, a process which again can be modulated by efflux transporters. Szakacs et al. [3] refer to the concerted interaction of metabolizing enzymes and efflux transporters as an effective chemoimmune system by which the body may be protected from adverse effects of xenobiotics.

Excretion processes are those by which compounds are ultimately removed from the body. A major route of elimination is renal excretion, but excretion into sweat, feces, and expired air are also possible routes. The percentage of urinary excretion (% exc), usually refers to direct renal elimination of unchanged compound. Excretion into breast milk raises specific concerns of potential toxicity to newborns, particularly as this may be the sole food source for the infant; hence their exposure is relatively high. Milk:plasma ratios (m:p) are useful in determining relative concentrations in breast milk.

The rate at which a compound is eliminated from the body is referred to as the rate of clearance (Cl). This is defined as the volume of blood completely cleared of compound in a given time. Total clearance is that by any route but renal excretion and hepatic metabolic routes predominate.

Half-life ($t\ ^1/_2$) is the time taken for the amount of compound in the body to fall by half. It is arguably the most important property as it dictates for how long the compound persists in the body and therefore the timescale over which it may elicit therapeutic or toxic effects. Half-life is determined according to Eq. (10-3) given below:

$$t^1/_2 = 0.693 V_d / Cl \tag{10-3}$$

By definition, half-life is governed by the extent to which the compound distributes throughout the body (V_d) and the rate at which it is cleared (Cl).

In determining biological effect, it is often desirable to relate activity to the concentration–time profile of a xenobiotic within a given tissue. For this physiologically based pharmacokinetic models (PBPK) may be used. In such models the biological system is represented as a series of organs, about which information, such as volumes and blood flows are known. These data are combined with compound-specific parameters (such as tissue partitioning, clearance) enabling the

full time-course of the drug to be predicted in individual tissues. PBPK modeling and its use is discussed by d'Yvoire et al. [2].

From the above descriptions, it is clear that ADME plays a key role in determining the extent of overall effect any compound has on the body. Hence prediction of these properties has gained widespread interest in the quest to predict, more accurately, both therapeutic and toxic effects of xenobiotics. Table 10-1 provides a summary of key ADME properties, along with reviews/example models for their prediction [6–39]. Several of these models are discussed in more detail below.

Table 10-1. Summary of key ADME properties and references for reviews/example models for their prediction

Properties	Definition	References
% Abs; % HIA	Percentage of available compound that is absorbed across a barrier; percentage that is absorbed across the human gastrointestinal tract	[6–9]
Skin permeability (K_p)	Permeability of a solute through skin, determined by flux measurements	[10, 11]
F	Bioavailability – fraction of dose that enters the systemic circulation	[9, 12, 13]
% PPB; f_b; f_u	Percentage of compound bound to plasma proteins; fraction bound to plasma proteins; fraction unbound (i.e., free fraction)	[14, 15]
V_d; V_{du}	Apparent volume of distribution, i.e., the hypothetical volume into which a drug distributes; V_{du} is the volume of distribution for the unbound fraction of drug	[16–20]
Tissue:blood PCs	The ratio of concentrations between blood and tissue	[21, 22]
BBB partitioning	Blood–brain barrier partitioning, frequently expressed as ratio of concentrations between brain and blood (serum/plasma) or expressed in binary format to indicate likely or not likely to enter brain	[9, 23–26]
CI; TI	Clearance index; transfer index for placental transfer of compounds usually expressed as a ratio using antipyrine as a marker	[27]
Transporter substrates/non-substrates/inhibitors	Relates to the affinity of compounds for a wide range of transporters, (several of which are defined in the text)	[28–30]
% exc	The percentage of compound excreted unchanged in urine	[31]
m:p	The ratio of concentration between breast milk and plasma	[32]
Metabolism	The process by which xenobiotics are converted to an alternative compound (usually one which can be more readily excreted). Of significance is the nature of the metabolite, the enzyme responsible for the catalysis of the process and the rate at which it occurs	[33–36]
Cl; Cl_{tot}; Cl_h; Cl_r	Clearance, i.e., the volume of blood from which a compound is completely removed in a given time; clearance by all routes; clearance by hepatic route (i.e., metabolism); clearance by renal route (i.e., urinary excretion)	[37]
$t_{1/2}$	Half-life, i.e., the time taken for the concentration of a compound in the body to fall by half	[38, 39]

10.1.2. Data for Generation of in Silico Models

As with all areas of model development, as more data become available, greater opportunity arises to produce more accurate and robust models. In terms of predicting ADME properties, the majority of data has been generated for pharmaceutical products. Whilst, undoubtedly, hundreds of thousands of compounds have been screened in drug development projects, unfortunately the majority of these data are proprietary and therefore not publicly available for modeling (a similar situation is described with respect to toxicity data in Chapter 11). This means that the publicly available ADME data, and hence models, tend to be skewed toward that minority of candidate compounds which exist in pharmacokinetically viable space. This is because all commercially available drugs will have acceptable, although probably not ideal, pharmacokinetic properties. When generating models it is better to have unbiased data sets with uniform coverage of the parameter space.

An additional problem of the bias toward commercially available drugs is that efforts to predict toxicokinetics of, for example, environmental pollutants are severely hampered by the paucity of accessible data. Models generated from pharmaceutical data may not be suitable to predict the ADME properties for a wide range of organic compounds, such as industrial chemicals and pesticides. Consideration of the applicability domain of a model is critical in terms of selecting the most appropriate model for a given query. Many publications are available for a detailed discussion of applicability domain, e.g., Netzeva et al. [40] and therefore this will not be considered further here.

With the increased interest in generating predictive ADME models, there has been a corresponding increase in the number of relevant data sets published in recent years. Data mining, i.e., collating and structuring data, from either in silico repositories or literature publications can provide valuable information. Table 10-2 provides a list of potentially useful data sets and the references [10, 11, 13, 15, 18, 21, 22, 24–27, 30, 32, 36, 37, 41–50] from which the full data are available.

Table 10-2. Examples of resources for ADME data

Properties	Information available	Reference
Human intestinal absorption	Data for 648 chemicals	[41]
Human oral bioavailability	Data for 768 compounds	[42]
Human oral bioavailability	Data for 302 drugs	[13]
Skin permeability	K_p data for 124 compounds	[10]
Skin permeability	K_p data for 101 chemicals	[11]
Protein binding data	Percentage bound to human plasma protein for 1008 compounds	[15]
Volume of distribution	Data for 199 drugs in humans	[18]
Tissue:air partitioning	Data for 131 compounds partitioning into human blood, fat, brain, liver, muscle, and kidney (incomplete data for certain tissues)	[22]

Table 10-2. (continued)

Properties	Information available	Reference
Tissue:blood partitioning	Data for 46 compounds partitioning into kidney, brain, muscle, lung, liver, heart, and fat (incomplete data for certain tissues)	[20]
Air:brain partitioning	Human and rat air–brain partition coefficients for 81 compounds	[21]
Blood–brain partitioning	Blood/plasma/serum/brain partitioning data for 207 drugs in rat	[43]
Blood–brain partitioning	Log blood–brain barrier partitioning values for 328 compounds	[24]
Blood–brain barrier penetration	Binary data for 415 compounds (classified as blood–brain barrier penetrating or non-penetrating)	[25]
Blood–brain barrier penetration	Binary data for 1593 compounds (classified as blood–brain barrier crossing or non-crossing)	[26]
Placental transfer	Placental clearance index values for 86 compounds and transfer index values for 58 compounds	[27]
Clearance	Data for total clearance in human for 503 compounds	[37]
Metabolic pathways	Catalogue of all known bioactivation pathways of functional groups or structural motifs commonly used in drug design using 464 reference sources	[44]
CYP metabolism	List of 147 drugs with the CYP isoform predominantly responsible for their metabolism (CYP3A4, CYP2D6, and CYP2C9)	[36]
Clearance; plasma protein binding; volume of distribution	Total clearance, renal clearance, plasma protein binding, and volume of distribution data for 62 drugs in humans	[45]
Milk:plasma partitioning	Concentration ratio data for 123 drugs	[32]
Transporter data	117 substrates and 142 inhibitors of P-gp; 54 substrates and 21 inhibitors of MRP2; 41 substrates and 38 inhibitors of BCRP	[46]
PgP data substrates and non-substrates	Binary classification of 203 compounds as P-gp substrates (+) or non-substrates (−)	[30]
% urinary excretion; % plasma protein binding; clearance; volume of distribution; half-life, time to peak concentration, peak concentration	A compilation of ADME data for approximately 320 drugs (incomplete data for some drugs)	[47]
Half-life; therapeutic, toxic, and fatal blood concentrations	Data for over 500 drugs (incomplete data for some drugs)	[48]
Volume of Distribution; % plasma protein binding; % HSA binding (from HPLC retention data)	Data for 179 drugs (incomplete data for percentage plasma protein binding for some drugs)	[49]
Toxicogenomics micorarray data	Gives literature references for data on 36 compounds	[50]

10.2. MODELS FOR THE PREDICTION OF ADME PROPERTIES

For the ADME properties defined in Section 10.1.1 above, a range of in silico models have been developed. Table 10-1, along with a summary of key ADME properties provides references to example models, or broader reviews of models, available for each property. It is not possible here to provide a detailed review of all the models or modeling approaches available. Therefore, the discussion below will provide an overview of selected models and approaches. The reader is referred to the given reviews for further information.

Some of the simplest models in predictive ADME are those referred to as "rules of thumb." The most widely recognized of these is Lipinski et al's "rule of 5" [6]. This was devised to provide a screening tool for compounds that were likely to show absorption problems, i.e., poor absorption is more likely if

- molecular weight > 500:
- sum of OH and NH hydrogen bond donors > 5:
- sum of O and N hydrogen bond donors > 10:
- C log P > 5:

This type of tool found ready acceptance amongst users because of its simplicity and ready interpretability. It has led to an increasing number of rules of thumb being devised for other endpoints. In 2002 Veber et al. [12] proposed a model for predicting good bioavailability, i.e., good bioavailability is more likely for compounds with:

- $\leq$ 10 rotatable bonds;
- polar surface area $\leq$ 140 A^2; or
- sum of hydrogen bond donors and acceptors $\leq$12.

Norinder and Haberlein [23] proposed two rules of thumb for determining whether or not a compound is likely to cross the blood–brain barrier. These are:

- if number of N+O atoms is $\leq$ 5, then it is likely to enter brain;
- if log Kow – (N+O) is positive, then log BBB partition coefficient is positive.

Developing the theme of rapid screening for large corporate libraries, Lobell et al. [7] devised a traffic light system for "hit-selection." In their approach five "traffic lights" are calculated for ADMET properties relevant to absorption through the gastrointestinal tract. The requirements for a compound to be well absorbed are that it is reasonably soluble, not too polar, lipophilic, large, or flexible. These factors are determined by the solubility, polar surface area, C log P, molecular weight, and number of rotatable bonds. These properties are all readily calculable and the values for individual compounds are combined to give a traffic light value. From this the most promising candidates can be selected.

More recently in 2008, Gleeson [18] reported a series of ADMET rules of thumb for solubility, permeability, bioavailability, volume of distribution, plasma protein binding, CNS penetration, brain tissue binding, P-gp efflux, hERG inhibition, and inhibition of cytochromes CYP1A2/2C9/2C1/2D6/3A4. The influence of changing

molecular weight and log P may have on these individual ADMET properties was demonstrated, providing a key as to how each of these may be optimized in drug development.

Simple structural information can also be useful in predicting other ADME properties. For example, the route of drug metabolism is determined by the presence of specific functional groups. Identification of such groups, hence deduction of likely routes of metabolism allows prediction of metabolic pathways important for identifying metabolites and potential drug–drug interactions. Manga et al. [36] showed the utility of using a recursive partitioning method (formal inference-based modeling) to predict the dominant form of P450 enzyme responsible for the metabolism of drugs. The model made use of descriptors for molecular weight, acidity, hydrogen bonding strength, molecular dimensions and log P. The model correctly identified which was the predominant enzyme responsible for metabolism for 94% of 96 compounds in the training set and 68% of 51 compounds in the test set.

Quantitative structure–activity relationship (QSAR) modeling has also proved useful in predicting ADME properties, although their use is generally restricted to smaller more homogenous data sets. QSAR models have been developed for many of the individual component processes in ADME.

Of the absorption processes, human intestinal absorption has received the greatest attention. Hou et al. [9] provide a review of 23 models for this endpoint including the use of multiple linear regression, non-linear regression, and partial least squares (in addition to neural network, support vector machine (SVM) and other analyses). Dermal absorption is of importance for both pharmaceuticals and environmental pollutants. Lian et al. [10] performed a comparative analysis of seven QSAR models to predict skin permeability, but concluded that more mechanistic studies were needed to improve predictions for this property.

General and specific distribution processes have also been successfully modelled using QSAR. In general terms, a global indication of distribution within the body is indicated by the apparent volume of distribution. Models for this global property have been developed by Ghafourian et al. [16] and Lombardo et al. [17]. Volume of distribution is dependent on the extent to which a compound binds to both plasma and tissue proteins. Colmenarejo [14] reviewed models available to predict binding to plasma proteins in addition to proposing new models to predict binding.

At a more local level, distribution into individual tissues is important in determining whether or not a compound is likely to distribute to a given site where it may elicit a therapeutic or toxic effect. Several models have been developed to predict tissue:blood partition coefficients, such as that described by Zhang [20]. In this model differential distribution into kidney, brain, muscle, lung, liver, heart and fat (based on tissue composition) was determined using 46 compounds. Of the tissue distribution models, partitioning into the brain has been the most extensively studied tissue. Hou et al. [9] provide a detailed review of 28 models to predict blood:brain barrier partitioning. More recently Konovalov et al. [24] using 328 log BBB values, proposed a system to benchmark QSARs for this endpoint to enable better comparison of current and future models.

From a toxicological perspective, an area of increasing concern is the partitioning of drugs and toxicants into the placenta as the potential to elicit toxic effects in the developing fetus is an important consideration. QSAR models to predict placental transfer of xenobiotics have been developed by Hewitt et al. [27].

HQSAR is a technique by which fragments of molecules are arranged to form a molecular hologram, such that three-dimensional information is implicitly encoded from input 2D structures. This technique was applied by Moda et al. [13] to the prediction of human oral bioavailability, providing reasonable correlations for this multi-factorial endpoint.

Three-dimensional modeling, although computationally more expensive, has also found a role particularly in binding studies, relevant to metabolism and determining affinity for efflux transporters. As discussed previously, in terms of metabolism, there is the potential for drug–drug interactions to occur between compounds that are metabolized by the same enzyme. Three-dimensional modeling of the specific interactions between ligands and their receptors provides greater understanding of the processes involved, which leads to improvement in predictions and helps to screen out such potential interactions during the design process. Three-dimensional QSAR modeling, pharmacophore generation, and homology modeling have all been applied to elucidate the role of P450s in drug metabolism. The application of 3D modeling to this field has been reviewed by de Groot [51] (Chapter 4). In particular, de Groot [51] discusses ligand-based and enzyme structure-based models. References are provided for pharmacophore models developed for P450s as well as references detailing known crystal structures for 15 bacterial, 2 fungal, and 7 mammalian P450s.

Determining which compounds are likely to act as substrates, non-substrates, or inhibitors for transport proteins is also important in predicting the overall internal exposure as well as tissue-specific exposure of xenobiotics. Chang et al. [28] provide a review of 3D QSAR studies for a wide range of membrane transporters including P-gp. Other studies on P-gp demonstrate another modeling technique which is proving useful in ADME modeling, i.e., the support vector machine (SVM) approach. Using this technique, Huang et al. [29] developed a model capable of distinguishing P-gp substrates from non-substrates with an average accuracy of over 91%.

Scientific opinion remains divided on the utility of neural networks to predict ADME and other endpoints. On the one hand, the flexibility of the approach enables non-linear relationships to be modelled. On the other, models are deemed to be non-transparent and difficult to interpret. However, many examples are available for the application of neural networks to this area. In terms of ADME models for excretion, one area of particular concern is the ability of a compound to be excreted into breast milk and the risk this may pose to neonates. Agatonovic-Kustrin et al. [32] used neural network methodology to develop a model identifying molecular features associated with transfer of drugs into breast milk. Turner et al. [45] also gives examples of neural network models for the prediction of total clearance, renal clearance, volume of distribution and fraction bound.

Whereas much of the above discussion relates to the development of individual models, one technique which appears to be growing in popularity is the use of consensus models. Banik [52] argues that as all models are simulations of reality, and therefore not totally accurate, a combination of individual models into a single consensus model can improve accuracy. An overview of different types of consensus models is presented, along with examples of where this approach has been shown to improve prediction accuracy.

The aim of the above discussion was to present an indication of the range of in silico modeling approaches available and information on where these have been applied to specific endpoints. However, within the literature there are also several reviews which cover the general application of in silico techniques to ADME predictions, as well as including comment on the status of the science. There are several examples of other useful reviews. Ekins et al. [53] provide an extensive review of available models and data sets. Duffy [54] gives an overview of models available and discussed selection of the most appropriate models for a given query; Gola et al. [55] review recent trends in predictive ADMET and argue for greater acceptance of in silico predictions, highlighting the importance of generating this data alongside activity data. Chohan et al. [56] draw on 61 references to review the status of QSARs for metabolism. Payne [35] provides an extensive review of techniques to predict metabolism. Winkler [57] discusses the role of neural networks in developing ADMET models. Dearden [58] reviews progress in the area of in silico ADMET modeling and provides a vision for future development in this area.

10.3. SOFTWARE DEVELOPMENTS

Increased awareness of the importance of ADME in modulating both therapeutic and toxic effects of xenobiotics has led to an increased demand for software to predict these properties. Software providers have responded to this demand and there now exists a comprehensive range of computer packages to predict ADME properties. Table 10-3 lists some of the software available for the prediction of ADME properties and provides a brief description of functionalities available within the programs.

Table 10-3. Software for the prediction of ADME properties and commercial databases

Software provider	Software package	Predicted ADME properties	Websites
Accelrys	Discovery Studio ADMET	Absorption, BBB penetration, plasma protein binding, CYP2D6 binding Physicochemical properties/toxicity	http://www.accelrys.com
Bioinformatics and Molecular Design Research Centre	PreADME	Physicochemical properties; permeation through MDCK and Caco-2 cells; BBB permeation; human intestinal absorption; skin permeability; plasma protein binding	http://www.bmdrc.org/04_product/01_preadme.asp

Table 10-3. (continued)

Software provider	Software package	Predicted ADME properties	Websites
BioRad	Know-it-All	Bioavailability, BBB permeability, half-life, absorption, plasma protein binding, volume of distribution, rule of 5 violations Physicochemical properties	http://www.knowitall.com
Chemistry Software Store	SLIPPER	Physicochemical properties, absorption	http://www.timtec.net/software/slipper/introduction.htm
ChemSilico	CSBBB CSHIA CSPB Other modules	BBB partitioning Human intestinal absorption Plasma protein binding Physicochemical properties/toxicities	http://www.chemsilico.com
CompuDrug	MetabolExpert MexAlert Rule of 5 Other modules	Metabolic fate of compounds Likelihood of first-pass metabolism Calculates rule of five parameters Physicochemical properties/ toxicities	http://www.compudrug.com
Cyprotex	Cloe® PK	Simulates concentration time course in blood and major organs; predicts renal excretion, hepatic metabolism and absorption; integrates experimental data	http://www.cyprotex.com
Laboratory of Mathematical Chemistry, Bourgas University	TIMES	Metabolic pathways	http://oasis-lmc.org/?section=software&swid=4
Genego	MetaDrug	Platform for the prediction of drug metabolism and toxicity	http://www.genego.com/metadrug.php
Lhasa	Meteor	Metabolic fate of compounds	http://www.lhasalimited.org/
Molecular Discovery	Metasite Volsurf+	Metabolic transformations Absorption, solubility, protein binding, volume of distribution, metabolic stability, BBB permeability	http://www.moldiscovery.com/index.php
MultiCASE	META/METAPC MCASE ADME module	Metabolic transformations Oral bioavailability, protein binding, urinary excretion, extent of metabolism, volume of distribution	http://www.multicase.com/products/products.htm
PharmaAlgorithms	ADME Boxes	P-gp substrate specificity; absorption; bioavailability; plasma protein binding; volume of distribution Physicochemical properties	http://www.pharma-algorithms.com/

Table 10-3. (continued)

Software provider	Software package	Predicted ADME properties	Websites
QuantumLead	q-ADME	Half-life, absorption, Caco-2 permeability, volume of distribution, humans serum albumin binding Physicochemical properties/toxicity	http://www.q-lead.com/adme_pk
Schrodinger	Qik Prop	Caco-2 and MDCK cell permeability, BBB permeation, serum albumin binding Physicochemical properties	http://www.schrodinger.com
SimCYP	Simcyp®	Population-based ADME simulator allowing profiles to be predicted in virtual populations [63]	http://www.simcyp.com/
Simulations Plus	ADMET Predictor	Intestinal permeability, absorption, BBB permeation, volume of distribution, plasma protein binding Physicochemical properties	http://www.simulations-plus.com
	GastroPlus	Physiological models for different species; dosage form effects; 1, 2, and 3 compartment models; complete PBPK models	
Provider	**Package**	**Commercial Databases**	**Websites**
Sunset Molecular	Wombat-PK	Database containing >6500 clinical pharmacokinetic measurements for 1125 compounds (bioavailability, percentage excretion, percentage plasma protein bound; clearance; volume of distribution, half-life, BBB permeation; metabolizing enzymes)	http://www.sunsetmolecular.com/
University of Washington	Metabolism and transport drug-interaction database	Database for enzyme and transporter interactions	http://www.druginteractioninfo.org/

The capabilities of these packages are diverse and are suitable for answering a variety of different queries. Facilities to predict key ADME properties, e.g., absorption, blood–brain barrier partitioning, and percentage plasma protein binding are available in many programs and require only the compound's structure for input. Examples include ADME boxes, ADMET predictor, Know-it-All, etc. (refer to Table 10-3 for more examples). Other software, such as Cloe®, requires the input of measured data (such as percentage plasma protein binding) in order to develop more accurate and comprehensive physiologically-based pharmacokinetic (PBPK) models of internal exposure. Within populations, different responses to xenobiotics are anticipated due to age, sex, health status, and genetic predisposition of individuals.

Simcyp® is a population-based ADME simulator that combines information from in vitro systems, drug-specific physicochemical information, and demographic, physiological, and genetic information to simulate ADME processes for drug candidates within a population. This provides a more mechanistic interpretation of pharmacokinetic or toxicokinetic behavior enabling predictions for subgroups of the population. Rapid expansion in the number of software packages available and their increasing sophistication is one of the hallmarks of recent advances in in silico tools for ADME prediction.

10.4. SELECTING THE MOST APPROPRIATE MODELING APPROACH

The information provided above demonstrates that a wide range of literature models and computational tools for predicting ADME properties is currently available and continuously expanding. The models range from high-throughput initial screening rules to detailed three-dimensional enzyme binding analyses, requiring a high level of computational power. As with all QSAR studies, the selection of the most appropriate model to use is dependent on the nature of the query, i.e., what level of detail is necessary to answer the question posed and which model is most readily interpretable and useful to the user.

Simple screening tools such as Lipinski's "rule of five" [6] or Gleeson's rules of thumb [8] have perennial appeal because of their simplicity. Such models relying on cut-offs for simple molecular features are readily interpreted by end users and easily acted upon. For example in drug design, knowing that drugs with a molecular weight above 500 may be associated with absorption problems, compounds can be designed with a maximum molecular weight of 450 Da, allowing for subsequent structural modification in the later optimization stages. Such approaches work well in certain circumstances, such as when a large diverse library of compounds is available and the aim is simply to begin to narrow down the number of potential drug candidates, or in priority setting where large numbers of compounds need to be considered. It must be noted, however, that these are simplistic models and inevitably there will be exceptions. The degree of inaccuracy acceptable is dependent on the purpose of the study. Whilst it may be acceptable to prioritize only those drugs with molecular weights below 500 Da in drug design, it would not be acceptable to presume that a toxicant present in food would not exhibit oral toxicity simply because its molecular weight indicates it may be associated with poor absorption characteristics.

Within a given series of compounds, a traditional QSAR approach can work well. Many QSAR models are available to choose from, but it is essential that the query compound falls within the applicability domain of the given model. Whilst more quantitative and detailed information may be available from these models, they tend to be of more limited applicability than the global screening methods. Global QSARs covering large numbers of diverse compounds have been developed, but there is a danger that reasonable global statistics may obfuscate poorer statistics for subgroups of compounds. This presents a real danger of misprediction if the query compound falls within this category [59]. It is advisable to ascertain the

reliability of the model to predict the required properties within the region of chemical space under investigation. One way of addressing this issue is by the use of "trainable" models. In this approach commercial or proprietary models are continually updated or re-trained using new data as it becomes available. The ADME Boxes approach from PharmaAlgorithms is an example of commercial software with this functionality. The chemical space within proprietary databases is unlikely to be fully represented by the chemistry used to train commercial models. It is important therefore that this additional knowledge can be captured and utilized to improve predictions in the chemical space relevant to the user. The region of chemical space under investigation at any given time is not a constant entity. The importance of allowing models to evolve over time has been exemplified by the study of Rodgers et al. [60]. In this study the authors demonstrated that models that were updated with new information over a two year period were more predictive than static models that did not evolve over time and advocated the "autoupdating" of QSAR models.

Once the nature of the query becomes more precise, a more specific and informative modeling tool may be required. For example, if detailed information is required on whether or not a drug may interact with a specific enzyme and therefore promote drug–drug interactions, three-dimensional modeling tools may become necessary. Another consideration in the selection of the most appropriate model is the selection of the most appropriate endpoint. For example, models exist to predict half-life of compounds; however half-life itself is a composite parameter based on volume of distribution and clearance. It is difficult to appreciate the influence of each of these individually from a global model for half-life. What may be more appropriate is to use individual models for volume of distribution and clearance, and then consider how these factors, acting in concert, may influence overall half-life. A similar argument can be made for the prediction of bioavailability which a composite parameter based on absorption, metabolism, and affinity for transporters. Features dictating the extent of absorption may not be the same as those dictating extent of metabolism or transporter affinity, but it is the combination of all of these parameters that controls overall bioavailability.

Figure 10-1 summarizes the different levels of information provided by the various modeling approaches and how this information can be fed into overall predictions of ADME behavior.

10.5. FUTURE DIRECTION

Overall, the application of in silico predictive methods to the area of ADME has shown much success in recent years. It is anticipated that this will be a subject of continual development in future not only in drug design applications but also in the area of predictive toxicology. This is because a better knowledge of the internal exposure of xenobiotics provides greater accuracy and understanding in the prediction of any biological effect. Being of relevance to both toxicology and drug development, affords the opportunity to address issues in predictive ADME from different perspectives and allows for a cross-fertilization of ideas. In drug design there are benefits in selecting more appropriate candidates to take forward

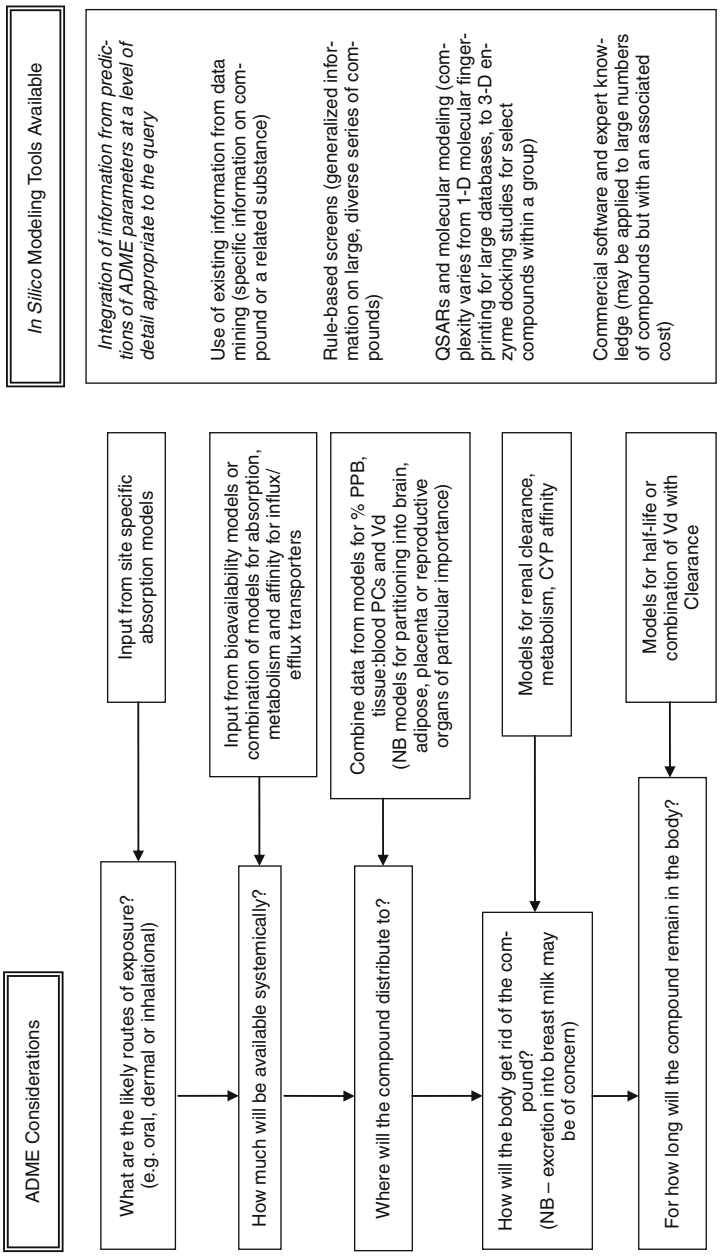

Figure 10-1. Flow diagram for incorporation of in silico ADME information into predictions for biological activity

(Chapter 9), whereas in toxicological risk assessment the science may be applied, for example, in priority setting for the testing of industrial chemicals (Chapter 11). Such data could be used to feed into integrated testing strategies and help determine the most appropriate testing protocols.

Previous, more naïve, concepts of biological systems have now been superseded by more mechanistic understanding. For example, uptake of a compound is not considered to be solely a passive absorption issue, but reliant on the orchestration of an entire chemoimmunity system of alternative absorption pathways, transporter affinities and integrated metabolic processes. The systems biology approach is providing more understanding of the interaction between xenobiotics and organisms as a whole [61]. As more is understood about the inter-relatedness of the biological pathways, the need to develop models for the individual processes becomes clear. The next step is the rational integration of all of these individual predictive models to enable the overall ADME behavior of a compound to be accurately predicted.

There are many opportunities for improvements in this field in the future. Predictions are currently limited due to a lack of high-quality experimental data; increasing the availability of this data will be crucial to model development. Within drug companies, an enormous amount of data has no doubt been generated on ADME properties of drug candidates; however, this is not publicly available. Information is also severely lacking in terms of ADME information for industrial chemicals and this presents a real challenge for the development of useful models for this type of compound. One potential way forward would be via the use of an "honest broker" as proposed by Dearden [58]. The broker could liaise with industry and enable proprietary data to be incorporated into the model development process in a secure environment. Increased availability of data would enhance the capacity to build more robust models. Current models for ADME properties tend to be skewed toward pharmacokinetically viable compounds as information is publicly available for these. Inclusion of information on the non-viable compounds would enable more balanced and robust models to be developed. A unified system for the collation and organization of available data would also be beneficial, but would require agreement on definitions and terminology.

In their discussion of why ADME models fail Stouch et al. [62] indicated that model transparency is a major factor. Model users need to be able to identify which compounds are used within the training set to ascertain if the model is suitable for their query compound (Chapters 5, 6, 12). Without this knowledge models can be criticized for performing poorly, but the reality is that the model may not have been suitable for the compound in question. Better relationships between model users and vendors could provide a mutually beneficial strategy in future. This approach has already seen success in the development of the DEREK for Windows software where a dynamic relationship exists between the software developers (Lhasa Ltd, Leeds) and the user group. Applying the same philosophy to ADME data may bring about corresponding improvements for this type of model. Similarly, extending the concept of trainable models, where in-house knowledge and expertise can be integrated into model development, is also a promising prospect.

Simultaneous development and integration of ADME predictions with the acquisition of knowledge on activity needs to become an intrinsic part of process in predicting the overall behavior of xenobiotics. This requires organizations to maximize collaboration and knowledge sharing between researchers in these areas.

10.6. CONCLUSION

For more than 30 years, drug development has benefited from in silico approaches to predict the activity and toxicity of drugs. Although, in general, researchers were slower at realizing the potential of these techniques to be applied to ADME predictions, this situation is now very different. One advantage of this is that many of the methods for in silico prediction have been rigorously investigated and developed for predicting activity, and the knowledge gained within that field can now be applied to the development of models to predict ADME. Many tools, using the state-of-the-art in silico methodology are now available to users and it is anticipated that the continual evolution of these tools will provide greater ability to predict ADME properties in the future.

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