18 Why Do We Need *In Vivo* Models in Drug Metabolism?

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18.1 SUMMARY

The use of preclinical *in vivo* models is integral to drug metabolism science. They are used to predict human pharmacokinetics in terms of oral absorption and distribution, including CNS penetration and drug clearance. In addition, *in vivo* models are essential in ensuring compound safety through qualification of human circulating metabolites in toxicology species.

The challenge in the use of *in vivo* models to predict human pharmacokinetics comes in the significant species differences in physiology and active processes (metabolism and transport) between animals and humans. Rat appears to be an appropriate model for the prediction of human oral absorption. For simple passive processes, such as volume of distribution and passive renal secretion, simple allometric scaling shows reasonable predictivity between animals and humans. For an indication of CNS penetration, the rat model has the greatest potential, although species differences in CNS penetration are known. However, when active processes, such as metabolism, biliary excretion, and renal secretion, are considered, species differences in rate and extent can significantly complicate extrapolation to human. These differences also preclude the use of preclinical models for the examination of human drug–drug and drug–food interactions.

As drug metabolism science progresses, much greater use of scalable human *in vitro* assays and *in silico* prediction should replace the use of animals in drug metabolism

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studies. Until that time, the use of *in vivo* animal models will remain essential in the drug metabolism field, although great care in their use should be exercised.

18.2 INTRODUCTION

A major aim of drug metabolism science is the description of the journey of a drug molecule through the body. It is important to understand how a molecule moves from its site of administration to the site of action, how long it remains there, and how it is eliminated from the body. These are termed the *absorption, distribution, metabolism, and elimination* (ADME) properties of a drug. It is the story of how the body deals with a drug molecule.

Since the inception of drug metabolism science as a separate discipline during the 1950s, scientists have developed a sophisticated understanding of how a drug molecule is handled by the body and, in many cases, can modulate the physicochemistry of a series of molecules to produce more desirable ADME properties (such as a longer elimination phase half-life) [1–4]. An understanding of the proteins that modulate the metabolism and pharmacokinetics of a molecule has been developed [5–8]. In addition, *in vitro* methods have been introduced, which further the understanding of drug metabolism and allow assessment of large numbers of compounds without recourse to *in vivo* preclinical models.

However, the use of *in vivo* animal models to understand drug disposition remains a key part of the drug discovery and development process. Wherever possible, a drug metabolism scientist has an ethical duty to replace *in vivo* experiments in preclinical species with *in vitro* and *in silico* approaches. However, with the current state of the science, a truly *in vitro* only approach to describe the properties of a molecule is not available. Thus, animals have to be used. The ultimate goal should be to replace all animal experiments with *in vitro* ones. However, until that time, the drug metabolism scientist is strongly recommended to follow a paradigm illustrated in Fig. 18.1, whereby

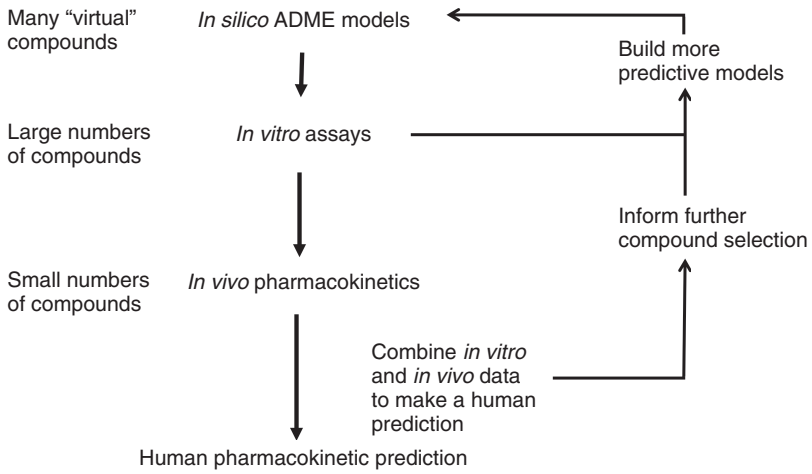

Figure 18.1 Proposed ADME screening funnel that drives to reduction in animal use.

large numbers of potential drug molecules are triaged using *in silico* and *in vitro* methods with only few compounds progressing to *in vivo* experiments.

From a pharmaceutical industry perspective, most drugs are aimed at the treatment of human disease. Thus, understanding ADME properties in humans is a key part of bringing a drug to the pharmaceutical market. A typical drug discovery and development programme (if successful) will take more than a decade to complete. Typically, medicinal chemists will synthesize thousands of molecules as putative drugs and the research process will reduce these to one or two for detailed scrutiny. During the development phase, the human safety and efficacy of these molecules are fully evaluated for presentation to regulatory authorities (such as the FDA). Drug metabolism science plays a significant role in the entire research and development process, with an interesting change in emphasis during the process. In early research, the aim is to identify pharmacologically active molecules with appropriate human ADME properties, without recourse to human *in vivo* experiments. Once the single compound has been selected, the drug metabolism effort tends to become descriptive with emphasis on a detailed understanding of the ADME profile of that molecule. In both phases, the use of *in vivo* models of drug metabolism is essential. However, there are significant species differences in the way drug molecules are handled from an ADME perspective. These species differences need to be well understood to enable correct decisions to be made.

The animal species used for drug metabolism *in vivo* experiments tend to depend on the phase of the project and the questions posed. In the research phase, the vast majority of animals used are rodents. *In vivo* pharmacology models tend to be in mice and rats and a significant proportion of drug metabolism effort can be expended in support of these models to understand PKPD relationships. As stated previously, the major aim of a research project is to select compounds with desirable ADME properties. Again, the *in vivo* model of choice is the rat, due to its relative availability and low cost. However, other nonrodent species (such as dog and nonhuman primate) are used during this phase. Whatever species is used, it is vitally important to understand the extrapolation of any pharmacokinetic property from the animal species to human. In the development phase, there is a regulatory requirement to complete toxicology studies in one rodent and one nonrodent species. Again the species most often employed are rat and dog or nonhuman primate. However, carcinogenicity studies are completed in mice and reproductive toxicity studies in rabbit. During the development phase, drug metabolism experiments are designed to support toxicology, ensuring that any chemical species derived from the drug in humans (i.e., metabolites) are also exposed to animals during toxicity studies (i.e., there are no human-specific metabolites). Thus, understanding of the metabolite profile of a molecule in mouse, rat, dog, rabbit, and nonhuman primate is important.

18.3 ORAL ABSORPTION

18.3.1 Oral Absorption and Bioavailability in Human

Most human drugs are administered at sites remote from the site at which they are designed to act. For patient ease of administration, the oral route is preferred. Orally administered drugs are most often given in tablet or capsule formulations. In order to be absorbed into the body, these solid formulations must disintegrate in the stomach and release their cargo of drug into aqueous solution in the gastrointestinal (GI) tract fluid.

The human GI tract is designed to be the organ of food digestion and nutrient absorption. The first organ encountered following oral administration is the stomach. This is where food digestion begins and can be a very acidic environment (pH 1–5) depending on the fed status of the individual. The major human drug absorbing site is the small intestine. This part of the GI tract is made up of three separate segments: the duodenum, jejunum, and ileum. In the small intestine, the pH returns to near physiological (pH 6.5–7.4). These segments contain a number of adaptations designed to facilitate the absorption of nutrients (and drug molecules), including luminal folding, villi, and microvilli, which increase the surface area for absorption. Finally, the lower part of the human GI tract is the large intestine (or colon), where the majority of the water involved in food digestion is reabsorbed before defecation.

Once a drug is in solution, it must pass across the GI tract wall to be absorbed into the body. There are two major routes of passage for drug molecules to pass across the GI tract wall: paracellular and transcellular (Fig. 18.2).

The major cellular barrier between the GI tract lumen and the blood is the gut wall epithelial cell or the enterocyte. To be absorbed, the drug must cross the enterocyte barrier. Between each enterocyte, there are very small tight junctions with aqueous pores through which low molecular weight compounds can be absorbed [9]. The estimated sizes of these aqueous pores in human small intestine vary from 5 to 13 Å, and there may be several size populations [10,11]. Drugs of small molecular weight can pass through these aqueous pores and are considered to be paracellularly absorbed. An example of such a drug is cimetidine [12], which is ~60% absorbed in human due to its molecular weight of 252 despite its hydrophilicity (cLog $D_{(7.4)}$ of −0.05). Owing

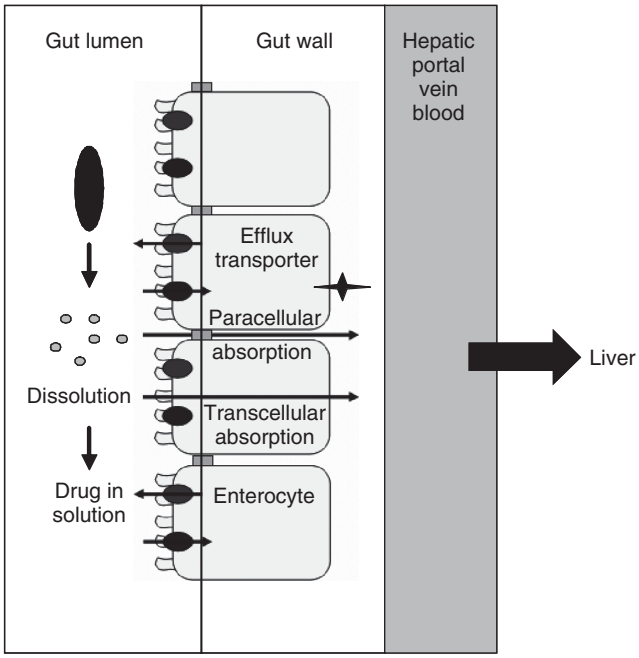

Figure 18.2 Schematic of the oral absorption process indicating paracellular and transcellular absorption.

to the size constraints and the relatively low surface area covered by these pores, the paracellular route is only a minor route of absorption for drug molecules. In addition, the aqueous pores become smaller lower down the GI tract and as such paracellular absorption from the colon is unlikely.

The major route of drug absorption is the transcellular route. To be absorbed by this route, the drug must dissolve in the apical membrane of the enterocyte and cross the cell to the basolateral membrane, cross that to enter into the blood. Drugs absorbed by the transcellular route are also subject to a battery of drug transporters that can either facilitate the absorption of the compound or act as a barrier to absorption by effluxing the compound back into the gut lumen. In addition, the gut wall cell contains many drug-metabolizing enzymes that can irreversibly remove the drug on passage through the cell.

Once a compound has passed through the gut wall, either by the paracellular or transcellular routes, it is considered to be absorbed into the body. However, it has one further barrier to overcome before it appears in the systemic circulation. All the blood from the gut passes into the hepatic portal vein, which flows directly to the liver. The liver is the major site of drug clearance and as such can exert a profound effect on the systemic concentrations of a drug in a process called *hepatic extraction*. The extent of hepatic extraction will depend on the physicochemistry of the drug and the affinity for drug transporters and metabolic enzymes. Owing to first-pass hepatic extraction, it is entirely possible that a compound can be completely absorbed from the GI tract but show no systemic concentrations due to complete removal by the liver. The extent to which a compound overcomes all the barriers between oral administration and the systemic circulation is called the *oral bioavailability*. It is a composite of the fraction absorbed from the GI tract and the fraction removed on first passage through the liver.

18.3.2 Animal Studies to Calculate Oral Absorption and Bioavailability

In the research phase, much emphasis is placed on the assessment of oral absorption in animal species as a predictor of human oral absorption. The most simple method to estimate oral absorption is by completion of an intravenous versus oral cross-over experiment in the species of choice. Following intravenous administration, all the dose is placed in the systemic circulation. Thus, the area under the plasma/blood concentration versus time curve (AUC) represents the complete dose. For an oral dose, the drug must dissolve in the GI tract contents and cross the GI tract wall (absorption). All the blood from the gut flows into the hepatic portal vein, which flows directly to the liver. The liver is a major drug extracting organ and, in order to reach the systemic circulation, the drug needs to avoid this potential "first-pass" extraction. Consequently, the AUC following an oral dose represents the amount of drug absorbed and the amount escaping first-pass extraction.

If it is assumed that clearance of a drug is solely by the liver, then the first-pass extraction of that drug can be estimated from the clearance of that drug and the hepatic blood flow in that species. Once the hepatic first-pass extraction is estimated, the AUC assuming complete oral absorption can be calculated. The ratio of the observed AUC to that expected following complete oral absorption represents the fraction of the dose absorbed in that species.

A more direct estimate of the fraction of an oral dose absorbed can be determined using the hepatic portal vein experiment. In general, this tends to be completed in

rodent species as it requires sampling of blood from the hepatic portal vein, thus eliminating the need to estimate hepatic first-pass extraction. Basically, the amount of dose absorbed can be estimated using one of two potential equations:

$$\text{Dose absorbed} = Q \times (\text{AUC}_{\text{portal}} - \text{AUC}_{\text{systemic}})$$

$$\text{Dose absorbed} = \text{Cl} \times \text{AUC}_{\text{portal}}$$

where Q is the hepatic portal vein blood flow and Cl the clearance of the drug.

18.3.3 Animal Studies as Models of Human Oral Absorption

The projection of extensive oral bioavailability in humans is often a key goal in preclinical drug discovery projects. Consequently, selecting compounds with appropriate oral absorption potential is important. There are significant differences in physiology between the human GI tract and those of animals used in *in vivo* drug metabolism experiments, which need to be understood for successful decision making. The use of animals for predicting human oral absorption has recently been reviewed [13].

18.3.3.1 Rat. The rat is the most studied preclinical model of human oral absorption. There are a number of physiological differences between the GI tracts of human and rat [14,15]. The pH in the rat duodenum tends to be slightly higher than that in human [15]. Clearly, the length and diameter of the human small intestine are higher than that in rat and there is significantly less unstirred water layer (the fluid nearest the gut wall epithelium) in humans than rats [14]. In addition, there are significant differences in terms of the bile system. Bile production in rat is some 16-fold higher than in human [16,17]. In addition, rat bile constantly flows into the GI tract, where it may have a solubilizing effect on low solubility agents. In contrast, all bile production in humans is stored in the gallbladder to be released following feeding. All these characteristics may lead to issues in terms of extrapolation of oral absorption between rat and human. However, in terms of aqueous pores, rat and human are similar with respect to size and distribution, which may suggest that the rat would be an appropriate model for paracellular absorption in humans [18,19].

Rat and human express similar amounts of drug transport proteins at the gut wall. Cao *et al*. [20] showed that several transport proteins (such as PepT1, MRP2, and GLUT5) are similarly expressed in rat and human intestine, whereas others (such as MDR1 and MRP3) are present in both species but at different expression levels. Indeed, the correlation between duodenal expression level of a large number of transport proteins in human and rat exhibited a strong r^2 value of 0.5687. There was a weak correlation between the rat and human expression level of intestinal drug-metabolizing enzymes.

Overall, Chiou and Barve [21] found a strong correlation between oral absorption in rats and humans for 64 drugs, which suggests that the rat is an appropriate model for human oral absorption, in the majority of cases.

18.3.3.2 Dog. A similar analysis of the dog suggests that it is not an appropriate model for human oral absorption under certain circumstances and should be used with caution in the research phase. The correlation between oral absorption in dog and human for 43 drugs was weak [22]. There are a number of potential physiological reasons for this.

In common with the rat, the intestinal pH is higher in the dog than in human. There is a higher bile salt secretion rate and a higher bile salt concentration that may drive to improved oral absorption of poorly soluble compounds [23,24]. In addition, the villus length in the dog small intestine is longer than that in human. Finally, total GI transit time is more rapid in dog than human. All these factors may play a role in the poor correlation between dog and human oral absorption. However, the major driver of this difference may well be the paracellular pathway.

The aqueous pore pathway in the dog assumes greater significance due to larger aqueous pores than human [19]. Many low molecular weight and polar compounds are absorbed by the paracellular route. In humans (and rats), there is a significant molecular weight cutoff with compounds >300 molecular weight exhibiting poor oral absorption by this pathway. However, in dogs, the molecular weight cutoff is higher, leading to significantly greater oral absorption for some compounds in the dog. For example, acyclovir (MW 225) shows <30% absorption in rat and human, whereas in dog, absorption is estimated at 85%. Similarly, atenolol (MW 266) is completely absorbed in dogs, but 22% and 55% absorbed in rat and human, respectively.

Unlike the rat, there appears to be limited information on expression of drug transporter proteins and metabolizing enzymes in the gut wall of the dog. However, species differences in oral absorption do not seem to be limited to paracellularly absorbed compounds in the dog. This is exemplified by the NK_2 antagonist UK-224,671 [25]. This compound would appear to be transcellularly absorbed as a molecular weight of 544 is unlikely to be absorbed through the aqueous pore pathway. It is a P-glycoprotein (P-gp) substrate [26], and this limits its transcellular absorption potential in mouse, rat, and human. However, in dog, the oral bioavailability is 55%, suggesting much greater absorption in this species.

18.3.3.3 Monkey. The use of monkey to predict human oral absorption has not been extensively studied. However, genetic and physiological similarities suggest that monkey may well be an acceptable model. The gastric pH is most like human than any other preclinical species. Gastric emptying time is similar to that in humans, whereas small intestinal transit time is similar to dog [27]. There is a paucity of data on expression levels of drug transporters and metabolizing enzymes in monkey gut.

Chiou and Buehler [28] have shown a good correlation between monkey and human oral absorption for 43 drugs, despite a range of solid dosage formulations being employed. Examination of solid formulations (tablets or capsules) is perhaps where the use of the monkey is most appropriate, since such formulations cannot be given to rats and the dog appears not to be a good model for human absorption. However, the balance of cost and ethics of monkey use needs to be strongly considered and use of this species should be strictly limited to fewer compounds at later stages of drug research.

18.3.4 Modeling the Effect of Food in Animals

A major consideration for human oral absorption is the effect of food on the absorption profile of a drug. The effect of food on the oral absorption of drugs in humans has been extensively reviewed for both general therapeutics [29,30] and for anticancer agents [31].

Food can have profound effects on both the rate and extent of drug absorption for certain compounds. For example, coadministration with food reduces the oral exposure of drugs such as doxazosin, verapamil, and zidovudine. In contrast, the absorption of felodipine, itraconazole, and phenytoin is increased by food. In addition, the rate of absorption is decreased by food for drugs such as diclofenac and paracetamol. Finally, there are significant numbers of drugs (including diazepam and amlodipine), where food has no effect on oral exposure in humans [30].

There are a number of potential reasons for these food effects on oral exposure. Food increases bile acid secretion and gastric retention, which will have an enhancing effect on poorly aqueous soluble agents and a potentially delayed effect on absorption rate. It may also increase hepatic blood flow such that high hepatic extraction drugs show reduced exposure after oral administration.

Given the potential variety of outcomes with food and the significant differences in GI physiology, modeling of human food effects in animals needs to be completed with great care. The rat should be ruled out as a model to predict food effects in humans due to its continuously high bile flow. It is also very difficult to achieve a truly fasted rat as on withdrawal of food, rats will tend to eat bedding or fecal pellets, meaning that there is always material in their stomachs. Dogs are not an appropriate model of human oral absorption in general as shown above. However, there are some accounts of the use of the dog in this context in the literature. For example, it has been shown that the food-driven 33–46% decrease in oral AUC of hydralazine in humans is adequately predicted by a food reduction in AUC of 63% in the dog [32]. However, celecoxib shows hardly any change with feeding in humans but a three- to fivefold increase in systemic AUC in the dog [33]. Finally, due to genetic and physiological similarities, the monkey may be an appropriate model, but there are very few examples of its use for studying food effects in the literature. It would appear that with state-of-the-art physiologically based pharmacokinetic models available (reviewed elsewhere in this book), the *in silico* approach to measure food effects in humans has largely superseded the preclinical study.

18.3.5 Oral Absorption Summary

Great strides have been made in the recent past in predicting human oral absorption in the research phase. Compounds with appropriate physicochemistry for oral absorption and acceptable *in vitro* absorption profiles will continue to be taken forward to preclinical animal species for oral absorption and bioavailability determination. The most appropriate species for this investigation is the rat based on its correlation with human oral absorption. The dog is not an appropriate model of human oral absorption and should be used with great care and a mechanistic understanding of the absorption process. Monkey may be an appropriate model but should only be used on rare occasions when solid dosage forms need to be investigated.

18.4 DISTRIBUTION

Distribution describes the reversible movement of a compound from the systemic circulation into tissues. It is dependent on the physicochemistry of the molecule driving a balance between plasma protein binding and tissue affinity [34].

18.4.1 Extrapolation of Animal Distribution to Human

The prediction of human volume of distribution has been extensively reviewed by Obach [34].

Since tissue distribution is driven by plasma protein binding and tissue affinity, extrapolation of the human volume of distribution from animal studies tends to be relatively straightforward. In general, tissue distribution is a passive process and tissues tend to show no real species differences (made of cells and membranes).

Therefore, it is possible to predict the human Vd of a particular drug using allometric (body weight) scaling [35]. The allometric exponent tends to approximate to unity [36,37]. However, in the early phase of drug research, the requirement for pharmacokinetics in three species to perform true allometric scaling can be limiting. Thus, many groups have tended to search for a single species that is as predictive for human Vd.

Obach *et al*. [38] examined several methods of prediction of human Vd. They found that allometric scaling of Vd (based on total drug) gave a relatively poor prediction of human Vd (53% of compounds with ±2-fold of actual human Vd). This improved significantly (77% within ±2-fold) if the unbound parameters were used. In addition, direct scaling of dog Vd corrected for species differences fraction unbound performed as well (81% within ±2-fold).

Other groups have examined alternative species for single species extrapolation of human Vd. Ward and Smith [39,40] used a correlation analysis to show that the monkey is an appropriate predictor of human Vd, whereas rat and dog showed less predictivity, based on total drug parameters. The same group recognized that the monkey may not be the most appropriate species to use in early drug research and have proposed incorporation of physicochemical rules to determine the likelihood of extrapolation errors from preclinical species [41]. Finally, Caldwell *et al*. [42] have shown that the extrapolation of rat Vd can exhibit a reasonable approximation of human Vd (average fold error 1.85 for 144 compounds with 84% of compounds with ±3-fold of actual human Vd), although this analysis does not factor in any species differences in fraction unbound between rat and human.

In summary, extrapolation of preclinical species Vd values is likely to provide a reasonable approximation of human Vd, especially when species differences in plasma protein binding are taken into account.

18.4.2 Free Drug Hypothesis

As described above, drug distribution is generally a passive process where there are no major barriers to drug diffusion into tissues. In these cases, eventually a steady-state situation arises, whereby the unbound concentration in blood reflects the unbound concentration in the tissues. Thus, for a large number of drugs, the unbound concentration in blood is a surrogate for the unbound concentration at the target. This is termed the *free drug hypothesis*.

18.4.3 Barriers to Passive Drug Movement

Within the body, there are a number of significant barriers to the passive movement of compounds between the blood and the tissue of interest. These include the

blood–eye and the blood–testis barrier. However, by far, the most important barrier is the blood–brain barrier (BBB).

The BBB acts as a highly selective barrier to the passage of compounds into the CNS. It is formed by the endothelial cells of the blood vessels supplying the brain. Unlike blood vessels supplying many other tissues, these cells form extremely strong tight junctions that effectively mean that the aqueous pore pathway does not exist. In addition, the membrane phospholipid make-up may be different from other endothelial cell membranes with a more rigid structure leading to increased difficulty in passive permeation. Finally, the cells express significant levels of efflux transporters and metabolic enzymes that are capable of removing compounds back into the blood on passage across the BBB.

By far, the most studied efflux transporter at the BBB is P-gp. Chen *et al*. [43] have extensively reviewed P-gp and P-gp knockout mice. The role of P-gp in preventing BBB penetration has also been extensively reviewed. The presence of P-gp as an efflux protein makes the BBB a formidable barrier for the passage of drug molecules. Molecules that are slow to permeate the endothelial cell membrane can be effectively removed and placed back into the blood by P-gp (and other efflux transporters). This has been exploited in the H1 antagonist area to provide drugs that show limited CNS penetration and thus the potential to exert a peripheral effect without the sedation side effects associated with central H1 antagonism. For example, fexofenadine is a potent H1 antagonist that is used in the treatment of allergic rhinitis. It is believed to be a substrate for P-gp and other drug transporters [44], and it is this aspect that prevents its entry into the brain and drives its lack of sedation activity versus earlier H1 antagonists [45,46].

In contrast, many drugs need to enter the CNS to exert their effects and so understanding brain penetration is a key feature for many drug research projects. This understanding often relies heavily on preclinical animal models.

18.4.4 Evaluation of CNS Penetration Preclinical Species

The evaluation of the potential of a compound to enter the CNS begins with the understanding of physicochemistry and the use of *in vitro* models of cell permeability. Promising compounds arising from these models are likely to be progressed to rodent studies. The major issues facing such studies are the route of administration and evaluation of which matrix to measure. Rodents are used because the end point is often cerebrospinal fluid (CSF) or brain tissue.

It is important to measure brain penetration, while plasma concentrations are as stable as possible. Following intravenous bolus administration, often the plasma concentrations decline rapidly during the early time points. Thus, brain concentrations may lag behind plasma concentrations at early time points, leading to an underestimate of CNS penetration (Fig. 18.3a). Following oral administration, the concentrations tend to rapidly rise and then fall as the compound is cleared. By far, the most appropriate route is the intravenous infusion to steady state (Fig. 18.3b), where the compound is infused at a constant rate for several hours, such that there is a steady rise in the plasma and potentially brain concentrations to a plateau, where the rate at which the compound is dosed is equivalent to the rate at which it is cleared.

The most simple measurement made in CNS penetration studies is the total concentration in the brain relative to the total concentration in the plasma (the brain to

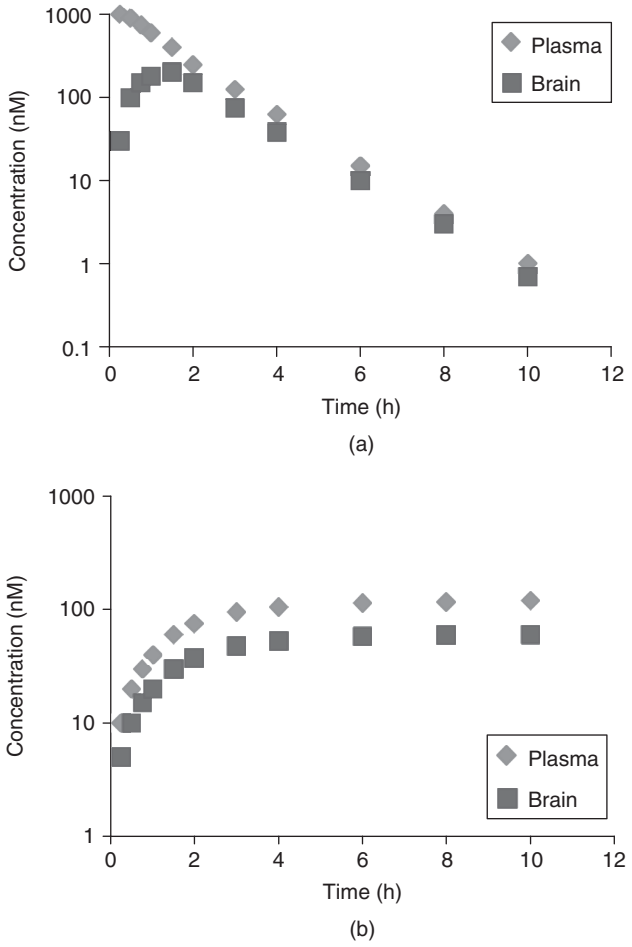

Figure 18.3 (a) Schematic of plasma and brain concentrations following an intravenous bolus administration assuming relatively slow CNS penetration. (b) Schematic of plasma and brain concentrations following intravenous infusion to steady state.

plasma ratio). Thus, at the end of the experiment, the animal is sacrificed and the brain removed. It is then homogenized and the brain homogenate analyzed for drug concentrations. This experiment assumes that binding to the brain tissue is the same as the binding to plasma proteins, and consequently, the free concentrations are equivalent. However, there are several issues with this approach. First, brain tissue is highly lipophilic and binding of lipophilic molecules is likely to be higher than that in the plasma. Thus, a compound with a brain to plasma ratio of greater than one, may reflect higher binding to brain tissue with the potential that unbound concentrations are low. Thus, a high brain to plasma ratio does not necessarily mean that the compound will have a therapeutic effect. Conversely, many lipophilic acid molecules bind tightly to plasma proteins with potentially less binding to brain tissue. Thus, total measurements may well underestimate the CNS penetration of such molecules.

The other potential issue with this experiment is that it is not possible to completely remove the blood from the brain before sacrifice. Since blood represents a small proportion of the volume of the brain tissue, compounds with high blood concentration may appear to have significant brain penetration.

Other groups have determined the extent of binding of a compound to brain homogenate and used this to calculate an unbound concentration in the tissue. This can be related to unbound concentrations in the plasma and is a more relevant measure of brain penetration and potential to achieve therapeutic concentrations in the CNS [47]. Thus, it is important to consider unbound concentrations in the CNS to fully define CNS penetration. The unbound concentration to measure would be the extracellular fluid (ECF), but this is a very difficult matrix to sample. Many groups have attempted to use microdialysis to sample ECF, but in the main, these have been hampered by the need for considerable surgical skill and the time-consuming experiments needed to quantify recovery of the probes. Another surrogate of the ECF is the CSF. This is a largely protein-free fluid that fills the ventricle in the brain. It is relatively easy to sample in the rat and can be compared to the unbound concentration present in the blood. The downside of this measurement is that the CSF concentrations may not necessarily reflect the ECF concentration, since there is a barrier between the CSF and the brain, which also expresses efflux transporters.

The other major issue with CNS penetration experiments in rodents as a model of human CNS penetration is that it is difficult to obtain good estimates of the human CNS penetration of a compound. Clearly, it is not possible to sample brain or complete microdialysis in humans, and the collection of CSF is challenging. The most appropriate method to achieve a measurement of CNS penetration in humans is to complete a PET imaging study, which requires significant investment and has not been completed on large numbers of compounds. Therefore, overall, it is very difficult to compile good data sets of CNS penetration in human for comparison with rodent experiments.

As a "rule of thumb," if a compound readily penetrates the CNS in a rodent, it is likely that it will readily penetrate the human CNS, making rodent studies the *in vivo* model of choice for CNS penetration. However, the issue with the use of the rodent is that there may be species differences in the efflux transporters at the BBB. In a recent study, species differences in the brain penetration of three P-gp substrates were examined by PET methodology [48]. This group found that verapamil, GR-205171, and altanserin penetrated the CNS of humans and monkeys more readily than rat brain. In addition, this species difference remained when P-gp was inhibited by administration of cyclosporin, suggesting that other efflux transporters or a unknown active process may be responsible for this difference.

18.4.5 Summary of Preclinical Models of Drug Distribution

The potential for a drug to distribute into tissues is an important consideration in the study of drug disposition. Distribution is a largely passive process determined by the physicochemistry of the drug. Tissues are largely made up of cells with membranes and so lipophilic molecules are more likely to distribute into tissues.

There are no major species differences in the composition of tissues. Therefore, preclinical species are appropriate models of drug distribution in humans. The most appropriate extrapolation to human involves the allometric (body weight) scaling of

the unbound parameter (i.e., corrected for any species differences in plasma protein binding) using an allometric exponent of 1.

The free drug hypothesis suggests that under equilibrium conditions, the unbound concentrations in the plasma reflect those in the tissues. Thus, if a research project is using an animal model of pharmacology, then the measured unbound concentration in plasma giving the desired effect should be close to the target efficacious concentration in humans (assuming no pharmacological potency differences).

The free drug hypothesis may not hold when the pharmacological target is behind a barrier to passive drug movement (such as the BBB). In this case, the ability of the drug to cross the barrier and the unbound concentrations in the tissue are of prime importance. In the absence of strongly validated preclinical models of human CNS penetration, rodent CNS penetration is likely to reflect that in human, so long as unbound concentrations are considered.

18.5 EXCRETION AND ELIMINATION

The human body is constantly renewing itself by turnover of proteins and other macromolecules. Similar turnover processes apply to drug molecules. Any administered drug is subject to removal from the body by a variety of processes. The removal of drug-related material (compound and metabolites) from the body is called *elimination*. The excretion of drug-related material is similar but usually refers to the organ that removes the drug (usually the liver and the kidney).

Drugs are removed from the body at different rates, depending on the rate at which they are acted on by excretion and elimination processes. The quantitation of the rate of removal of a drug from the body is an important consideration and is captured by the pharmacokinetic concept of clearance.

18.5.1 Allometric Scaling of Clearance

It follows that human clearance is an important parameter to be able to predict from preclinical information. This is because it in large part determines the exposure of a compound for a given dose.

The most widely used method of prediction of human clearance from preclinical pharmacokinetic information is allometric scaling. This attempts to relate the clearance of a molecule to the body weight of the species investigated. The basis of this scaling dates back to an early paper [49], which suggested that metabolic rates could be related to body size using an allometric exponent of ~0.75. Boxenbaum [50] developed this further by showing that physiological parameters such as liver weight and hepatic blood flow can be correlated to body weight with allometric exponents of 0.8–0.9.

The rationale behind simple allometric scaling is that clearance can be related to body weight using the following equation:

$$\text{Clearance} = \text{a} \times \text{body weight}^{x}$$

where a is a constant and x is the allometric exponent.

Thus, a plot of log clearance (mL/min) versus log body weight (kg) would be a straight line with a slope being the allometric exponent (Fig. 18.4).

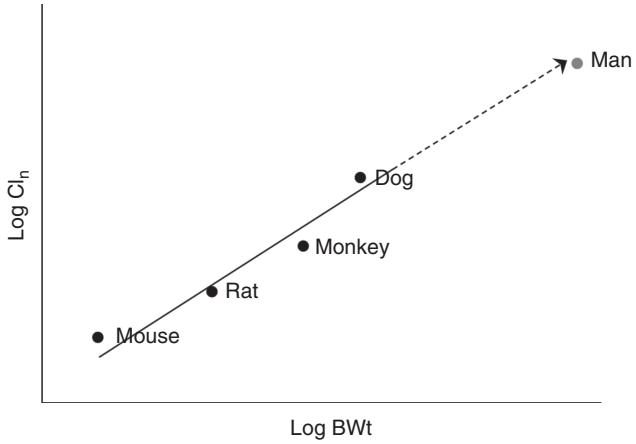

Figure 18.4 Principle of simple allometric scaling.

Using this method, the clearance of a compound in human could be predicted from extrapolation of the line to human body weight.

The success rate of simple allometric scaling in prediction of the human clearance of 102 molecules has been evaluated by Tang and Mayersohn [51]. The allometric exponents ranged from 0.26 to 1.2, although the majority were in the range 0.6–0.8. Overall, the average prediction error (APE) for this compound set was 254%. However, the authors noted that the application of simple allometry could be prone to some highly significant individual errors, in some cases over 1000%. Furthermore, they noted that the majority of these large errors seemed to occur in the low clearance (with respect to hepatic blood flow) cohort. Indeed, the low clearance cohort showed an APE of 598%, whereas the high clearance cohort showed an APE of only 47%. Their analysis suggested that a compound was more likely to be mispredicted to a large extent when the cLog P was >2 and the species difference in plasma fraction unbound was >5. They rationalized this finding on the basis that for high clearance agents, the major contributor to that clearance by the liver or kidney was the hepatic or renal blood flow. Since blood flows scale well allometrically, so will high clearance agents. In contrast, the major determinant of low clearance is the unbound intrinsic clearance of the molecule for the clearing process (likely to be metabolism or active transport). For these clearance pathways, as will be shown later, significant species differences exist that will introduce error to the allometric extrapolation. This would fit with the overall suggestion that passive processes are likely to scale well allometrically, whereas active processes are prone to species differences and therefore error in extrapolation.

In addition to the potential for significant prediction errors, there are a number of issues with simple allometry for the prediction of human clearance. First, it requires pharmacokinetic studies in at least three species and so it is not especially amenable to compound selection in early drug research. Second, it is nonmechanistic and does not take into account the clearance mechanism of a particular molecule nor the potential for species differences in the rate of active processes. Finally, although the initial idea was that clearance would scale with an exponent similar to that with which physiological processes scale, the exponents for simple allometric scaling of clearance can show

significant deviation from 0.7 to 0.8. Indeed, Mahmood and Balian [52] argue that there is no reason why the allometric exponent for a particular drug should be 0.7–0.8. These authors have shown that human clearance prediction is improved by the incorporation of factors depending on the allometric exponent. When the allometric exponent is between 0.55 and 0.70, simple allometry predicts most accurately. For exponents of between 0.71 and 0.99, the maximum lifespan potential should be incorporated, and for exponents above 1, clearance multiplied by brain weight is more accurate. For a more in-depth analysis of the use of allometry with the rule of exponents, the reader is directed to several papers by Mahmood [37,53].

To address the requirement for pharmacokinetic studies in multiple species, several groups have proposed methods of human clearance prediction using scaling from one single species. One of the most comprehensive evaluations has examined the hepatic blood flow method [39–41], whereby the human clearance is predicted from the product of the animal clearance and the ratio of the human and animal hepatic blood flow. For a series of 103 compounds, the monkey hepatic blood flow predicted human clearance more successfully than rat or dog or the use of simple allometric scaling. Although these authors concluded that use of the monkey method was quantitatively more predictive than other species, they recognized that use of the monkey in early drug research projects is problematic. For this reason, other authors have proposed the use of single species allometric scaling from rat using a fixed exponent of 0.75 as a pragmatic and reasonably predictive method for the prediction of human clearance [42,54].

The use of fewer species and the incorporation of fixed exponents single species scaling is highly controversial [55,56]. This author argues that allometric exponents have no physiological relevance. The use of a fixed exponent of 0.75 is misleading and prone to significant prediction error. As shown previously, the preferred method is multiple species allometry using the rule of exponents.

Irrespective of the literature controversy, it appears that the use of preclinical species to predict human clearance will continue. This is despite the fact that no universal method appears to have the required degree of accuracy to predict differences between two closely related compounds [57]. In addition, many of these methods can produce some highly misleading human clearance predictions (probably as a result of species differences in active clearance processes).

18.5.2 Clearance Processes

There are a number of ways a drug can be eliminated from the body. The major routes are by metabolism, via the bile and via the urine. In a survey of the top 200 marketed drugs [58], by far, the predominant route of elimination was by metabolism. The second major route was in the urine, with only small numbers of compounds being eliminated in the bile.

18.5.2.1 Metabolism of Drugs. In the same review [58], the enzymes responsible for the metabolism of the top 200 marketed drugs were investigated. By far, the most predominant enzyme responsible for metabolism is the cytochrome P450 (CYP) family. The next most prominent drug-metabolizing enzymes are the UDPGlucuronyl transferases (UGTs), followed by more minor contributions from aldehyde oxidase (AO), monoamine oxidase, and sulfotranferases.

18.5.2.2 Cytochrome P450 (CYP) Metabolism. The CYPs are a superfamily of drug-metabolizing enzymes that, in general, catalyze the oxidation of drug substrates. In the main, they cause the hydroxylation of carbon atoms and the dealkylation at heteroatoms such as nitrogen or oxygen. They are expressed in many tissues, but the greatest expression occurs in the liver.

The CYPs comprise a family of several hundred isoforms, but by far, the most important human drug-metabolizing CYPs are CYP3A4, CYP2C9, CYP2C19, CYP2D6, CYP2E1, and CYP1A2. Each has its own structure substrate metabolism relationship.

18.5.2.3 Preclinical Models of CYP Metabolism. There are a number of distinct species differences in the structure and function of the CYPs. The same isoforms are not expressed across the preclinical species [59].

Table 18.1 shows the significant differences in the expression and substrate specificities of CYP isoforms between human and animal species. These marked species

TABLE 18.1 Species Differences in the Expression of CYP Isoforms

CYP Subfamily	Species Extrapolation	Inhibition	Induction
CYP1A	Strong conservation across species	Human inhibitor furafylline shows variable inhibitor profile in preclinical species	Omeprazole induces in human but shows variable induction in animals
CYP2A	Significant species differences in substrate specificity	NA	Different inducers in animals and humans
CYP2B	Different isoforms with different substrate specificities	Similar inhibitor profile across species	Phenobarbital induces in all species
CYP2C	Sex-dependent expression in rats. Poor expression in dogs. Significant substrate specificity differences between animals and humans	Inhibition by sulfaphenazole in human, monkey, and dog. Limited inhibition in rat	Inducible in humans
CYP2D	Polymorphic expression in human	Inhibition by quinidine in human, monkey, and dog but not rodents. Inhibition by quinine in rat, dog, and monkey but not human	Not inducible
CYP2E	Relatively well conserved across the species	Similar inhibitory profiles	Inducible by ethanol and acetone
CYP3A	Markedly different substrate specificities across the species	Species differences in inhibition	Species dependency in induction

differences (depending on the isoforms in question) could lead to major differences in clearance by CYPs across the species. Given that a large proportion of marketed agents are cleared by CYP metabolism, it is perhaps not a surprise that allometric scaling based on body weight shows moderate predictivity, with the potential for large extrapolative errors [51]. For these substrates, human liver microsomal scaling may be a more appropriate clearance prediction method.

In addition, there are striking species differences in the CYP inhibition and induction profiles. For these reasons, CYP induction or drug–drug interaction (DDI) studies in animal species as predictors to human are not recommended.

18.5.2.4 UDPGlucuronyl Transferases. The UGTs are a family of drug-metabolizing enzymes that catalyze the addition of glucuronic acid at phenol, alcohol, acid, and nitrogen functions. There are a significant number of isoforms expressed in human liver, including UGT1A1, UGT1A3, UGT1A6, UGT1A9, UGT2B7, UGT2B10, and UGT2B17. In addition, there is significant expression of UGTs in human intestine that can contribute to reductions in oral bioavailability due to gut wall first-pass glucuronidation.

As for the CYPs, each UGT has its own structure substrate metabolism relationship. Unlike CYPs, there has been limited research into the SAR of UGTs. However, some species differences in glucuronidation are beginning to emerge in the literature. For example, the major human urinary metabolite of afloqualone is an *N*-glucuronide that is not present in the urine of animals. *In vitro* studies suggest that the rate of glucuronidation is significantly higher in human liver microsomes than in microsomes from rat, dog, and monkey liver [60]. In addition, mycophenolic acid was glucuronidated at a higher rate in human liver microsomes than in rat liver microsomes [61]. Finally, there is a significant species difference in the oral bioavailability of raloxifene (39% in rat and 2% in human). It has been suggested that this is driven by a 33- to 72-fold difference in the rate of 4-hydroxy glucuronidation between human and rat intestinal microsomes, leading to a much greater intestinal first-pass extraction in humans.

In common with clearance by CYP enzymes, these potential species differences in glucuronidation will complicate extrapolation of human pharmacokinetics from animals. Thus, for such active metabolism processes, allometric scaling may be seriously flawed.

18.5.2.5 Aldehyde Oxidase. Of the more minor enzymes contributing to metabolism of drugs, AO potentially exhibits the most marked species differences. This enzyme is a cytosolic molybdenum-containing protein that tends to oxidize substrates on heterocyclic nitrogen-containing groups.

There are a number of documented observations of significant species differences in AO expression and activity. These include a potential null polymorphism in Sprague-Dawley rats [62] and the total absence of activity in the dog [63]. In those species where AO is expressed, there appears to be significant differences in activity with monkey showing greater activity than rat [64]. Sahi *et al*. [65] suggest that the rank order of AO conversion of vanillin to vanillic acid is monkey > mouse > human > rat.

These species differences in activity follow through to *in vivo* pharmacokinetics. The clearance of SB-2777011 (an AO substrate) was low to moderate in rat and dog, leading to moderate oral bioavailability [63]. However, in monkey, clearance approximated to hepatic blood flow and oral bioavailability was 2%. *In vitro* investigations

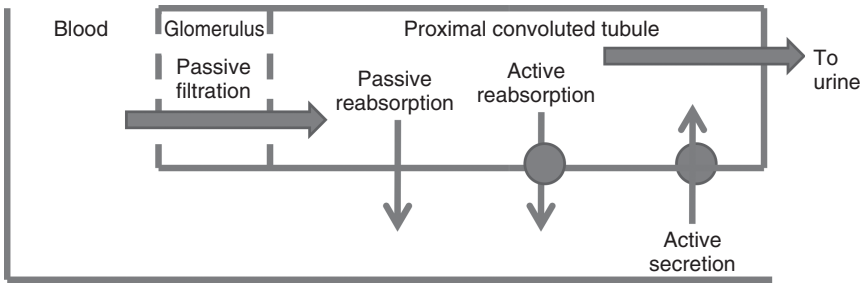

Figure 18.5 Schematic of renal excretion at the nephron.

suggested that human liver cytosol metabolized SB-277011 at an equivalent rate to that of monkey, suggesting the potential for low human oral bioavailability.

Overall, when AO is the principal contributor to the metabolism of a compound, care must be taken in the choice of species for human clearance prediction. The absence in dog and the low and polymorphic expression in rat suggest that these species are not suitable models for human AO clearance.

18.5.2.6 Renal Elimination. A second major human route of elimination is in the urine. Renal elimination of drugs has four distinct contributors (Fig. 18.5):

- Passive filtration of unbound drug at the glomerulus
- Transporter-mediated renal secretion into the urine from the blood
- Extent of passive renal reabsorption from the urine
- Transporter-mediated renal reabsorption from the urine

The amount of a drug excreted unchanged in the urine will depend on the balance of these four activities.

18.5.2.7 Passive Processes. Blood flows into the Bowman's capsule in the kidney where it is subject to filtration. Large components such as blood cells and plasma proteins do not pass through the filter and are retained in the blood. Small molecules (including drugs) that are not bound to plasma proteins are filtered into the proximal convoluted tubules.

For a compound that is not subject to active processes, the amount excreted in the urine will depend on how much of the drug returns back into the blood by passive diffusion across the epithelial cell of the proximal convoluted tubule. This, in turn, depends on the physicochemistry of the drug. Lipophilic drugs are likely to permeate across the membranes and be reabsorbed, leaving very little in the urine. Polar drugs have difficulty permeating membranes and consequently remain to be excreted in the urine. A good example of a passively renally cleared compound is a gabapentin, which shows essentially complete renal excretion at glomerular filtration rate (GFR; clearance 9.5 mL/min/kg) in rat [66] and human (clearance 1.8 mL/min/kg) [67].

Since the latter type of compound tend to be minimally plasma protein bound, their clearance tends to relate to the rate of blood flow to the glomerulus (i.e., GFR). Human GFR is ~1.5 mL/min/kg; consequently, the human clearance of gabapentin is

close to 1.5 mL/min/kg. In addition, because this is a passive process, it relates to GFR across the species. Since blood flows are allometrically related, the clearance of such molecules in preclinical species is often an accurate predictor of the human clearance.

A similar relationship holds for compounds that are predominantly passively renally cleared but show a significant degree of passive reabsorption. This is exemplified by fluconazole. Urinary excretion of unchanged fluconazole is the predominant route of elimination across all species. Fluconazole is also highly passively permeable and a constant 80% of the filtered material is reabsorbed from the proximal convoluted tubule. Thus, the renal clearance of fluconazole is ~20% of GFR across the species and scales extremely well allometrically from animals to human [68].

18.5.2.8 Active Processes. Some drugs are substrates for active transporters in the kidney. They can be extracted from the blood and effluxed across the proximal convoluted tubule epithelium by these drug transporters. Such compounds have renal clearances above that which is possible by GFR alone. A good example is lamivudine with 70% of the dose excreted unchanged in the urine in humans with a clearance of 20 mL/min/kg (i.e., greater than GFR [41]).

Conversely, some compounds are substrates for active reabsorption from inside the proximal convoluted tubule back into the blood. Such compounds show predominantly urinary excretion, but their clearance values tend to be significantly less than possible by GFR.

The kidney transporters are beginning to be elucidated and include organic anion transporters (OATs), organic cation transporters (OCTs), and organic anion transport proteins (OATPs) [7,8]. However, the preclinical transporters are not yet fully elucidated and (as with the CYPs) there are likely to be significant species differences in expression and activity. Therefore, allometric scaling of the clearance of such molecules may be problematic, suggesting that preclinical models may not be good predictors of human active renal excretion.

18.5.2.9 Biliary Elimination. Hepatobiliary elimination is a three-step process, most of which is driven by active transport. The first stage is uptake into the hepatocyte usually by drug transport proteins (such as OATPs) at the sinusoidal membrane of the hepatocyte. The compound is transferred across the hepatocyte, avoiding metabolism, to the biliary canalicular membrane. Active efflux into the bile is completed by biliary canalicular transporters, such as MRP2 and BCRP.

There are a number of species differences in the biliary elimination process. First, as with all active transport processes, there are species differences in the drug transporters involved, both in terms of expression and structure activity relationships. For example, it is thought that MRP2 expression at the rat canalicular membrane is significantly greater than that at the human canalicular membrane, whereas dog and monkey expression is similar to human [69]. In addition, the physiology of the rat biliary system is significantly different from human (and other species). Rat bile flow is some 16-fold higher than human [70]. In addition, rats do not have a gallbladder and bile continually flows into the gut lumen. In humans and higher preclinical species, the bile is stored in the gallbladder and is released into the gut lumen on feeding.

For these reasons, the rat tends not to be a good model of human biliary elimination. Some compounds can show high hepatic extraction in rat due to biliary elimination, which does not always translate to similar hepatic extraction in humans. A good

example of this is susalimod [71], where allometric scaling between preclinical species predicted relatively well, but there was a significant overprediction of human clearance (predicted 1.8 ml/min/kg vs actual 0.07 mL/min/kg). Indeed, allometric scaling of clearance by biliary elimination is fraught with difficulty and various scaling factors have been suggested [70].

The other major difficulty with the study of biliary elimination in humans is access to the bile. Sampling of bile in humans requires surgical cannulation of the gallbladder and so examples of true biliary elimination are limited. In addition, compounds with biliary elimination in rat tend to show significant hepatic extraction in this species. Simple allometric scaling of these types of compounds is likely to predict extensive clearance in humans, and so they may be selected out of the compound optimization process. Thus, these aspects may well explain the relatively few examples of biliary eliminated molecules in the top 200 marketed drugs list [58]. As transporter science evolves and understanding of biliary elimination is improved, this situation may change.

18.5.2.10 In Vivo Models of Human Clearance. A major driver for completion of *in vivo* pharmacokinetic studies in preclinical species is the prediction of human clearance. The most appropriate experiment is the intravenous administration of the compound followed by extensive blood or plasma sampling. The dose used is important as clearance can be saturable, so the dose needs to be in a similar range to that expected in humans. The plasma or blood clearance can be related to plasma or blood concentrations as shown previously. Comparison of blood clearance with hepatic blood flow will give an indication of the hepatic extraction of the compound (especially if the drug is solely cleared by the liver). The blood clearance (or more appropriately the unbound clearance in blood) can be allometrically scaled (as described previously) to gain a prediction of the human clearance of that molecule.

A further advantage of the preclinical *in vivo* pharmacokinetic study is the ability to collect other matrices and examine the fate of circulating metabolites of the compounds. The measurement of unchanged compound in urine will give an indication of the renal clearance. When compared with the GFR, such experiments yield the potential for a compound to be passively filtered or actively transported to or from the urine.

The determination of biliary elimination of unchanged drug cannot be determined from a simple *in vivo* pharmacokinetic study. The collection and measurement of bile requires surgical cannulation of the bile duct. For this reason, the rat is most often the species of choice. Pharmacokinetic studies can be conducted in bile duct cannulated rats, which has the advantage of relating plasma clearance to the biliary excretion rate. An alternative experiment that can be used to examine biliary elimination is the isolated perfused rat liver (IPRL). This has the advantage of enabling rat hepatic extraction as well as the biliary excretion of unchanged drug and metabolites to be determined. A good example of the use of the IPRL is shown in the examination of the disposition of 5,6-dimethylxanthenone-4-acetic acid, where unchanged drug accounts for 28% of the administered dose with the acyl glucuronide accounting for >50% of the dose [72].

Given the species differences in clearance and the variety of potential mechanisms by which a compound can be removed from the body, the use of preclinical species to model DDIs is fraught with difficulty. The choice of DDI perpetrator for any compound would be difficult, as would be the dose at which it should be administered. These facets are well established for humans and can be modeled using *in silico* techniques. Therefore, the study of DDIs in preclinical models is not recommended.

18.5.3 Metabolism and the MIST Guidance

One of the most important aspects of the use of *in vivo* models of drug metabolism comes in the drug development phase. It is essential that drugs are safe for administration to humans. This is achieved through rigorous toxicology testing in preclinical species. In these studies, it is extremely important that not only the unchanged drug is qualified in humans but also the metabolites of that drug. Thus, it is essential to show that any compound-related material that is exposed to humans has also been exposed to at least one toxicology species with no toxicological consequences. In general, this is achieved by administration of radiolabeled drug to the preclinical species used in toxicity testing. Assuming that the site of radiolabeling has been chosen appropriately, any circulating or excreted drug-related material will also be radiolabeled, and these components need to be identified and quantitated. Indeed, Metabolites in Safety Testing (MIST) guidance from the FDA and subsequent ICH M3 guidelines require any metabolites constituting more than 10% of drug-related material circulating in human plasma is qualified in each toxicology species. Thus, these types of *in vivo* studies represent a major component of the ADME package for a drug submission.

18.5.4 Summary of Preclinical Models of Clearance/Elimination

The clearance of a drug is an important parameter in humans as it relates dose to exposure. There are many ways by which a drug can be removed from the human body. Many drugs are cleared by metabolism with a significant proportion removed by active or passive renal excretion. In addition, biliary elimination of unchanged drug is another potential route. The active processes require intervention from proteins (enzymes and transporters) within the body. There are a number of species differences in terms of expression and structure activity relationships. This makes extrapolation of human clearance from preclinical animal models difficult.

Allometric scaling has been moderately successfully used to predict human clearance. However, it is likely that allometric scaling will work very well for passively cleared compounds and those that are cleared at close to hepatic blood flow (as these are dependent on blood flow and scale well with body weight). The issue comes in the extrapolation of clearance by active processes, where species differences will confound scaling on the basis of body weight and potentially lead to large errors in extrapolation.

At present, extrapolation of human clearance from preclinical *in vivo* models remains an appropriate method for most clearance pathways. The exception is CYP clearance where scaling of human liver microsomal experiments should be more appropriate. As drug metabolism science moves on, further scalable human *in vitro* models of clearance should be advanced that will reduce the need for *in vivo* animal models in this area.

18.6 PERSPECTIVE ON THE USE OF *IN VIVO* MODELS OF DRUG METABOLISM

The use of *in vivo* models has been integral to the progression of drug metabolism science. These models are used extensively to predict the human disposition of potential drug molecules and to support the progression of compounds through drug development, especially in support of toxicology studies. These *in vivo* models are likely to

TABLE 18.2 Summary of the Utility of Animal Models of Drug Metabolism for Predicting Human Drug Disposition

ADME Property	Model Species	Comments
Oral absorption	Rat correlates with human	Rat is an appropriate model for transcellular and paracellular absorption
	Monkey correlates with human	Paracellular pathway in dogs is larger
	Dog has a poor correlation with human	
Effect of food on oral absorption	No appropriate model for human	Rat physiology is different (bile flow). Difficult to fast rats. Dog precluded by physiology differences and paracellular pathway. Use *in silico* models
Volume of distribution	Scales well from all species, especially if fraction unbound considered	Passive processes scale well from preclinical species
CNS penetration	Rodent models appear best for human prediction. Must use unbound fraction in brain or CSF. Emphasis on which matrix to measure in rodent and human	*In vitro* systems should be used to triage compounds before *in vivo* evaluation
Metabolic clearance	Significant species differences. Will scale well for clearances approaching hepatic blood flow. For low clearance agents may be prone to errors due to it being governed by intrinsic clearance	For CYP substrates, human liver microsomal scaling may be a more appropriate predictor of human clearance. As scalable human *in vitro* systems become available, the use of animal models for human metabolic clearance prediction should reduce
Renal clearance	Passive renal clearance scales well from any preclinical species. Active secretion or reabsorption likely to be prone to species differences	For passive renal clearance, human clearance of unbound drug should approximate to human GFR. Prediction of active process should benefit from identification and expression of the transporters involved
Biliary clearance	Active process. Likely to be species differences. Physiology differences	Prediction of biliary clearance will continue to be problematic until human scalable *in vitro* assays become available
Drug–drug interactions	Likely to be poor predictivity due to species differences in clearance	DDI studies not recommended in animals. CYP DDIs are well predicted using *in silico* models

continue to be used into the future, but as drug metabolism science evolves, there needs to be continual scrutiny on their effective use. Every scientist who uses animals in his/her research has an ethical responsibility to minimize that use and replace with suitable *in vitro* alternatives. Drug metabolism has many future opportunities to do this.

A perspective of the current use of *in vivo* models of drug metabolism to predict human disposition is reviewed in Table 18.2.

It is clear that there are many issues with prediction to human and a number of approaches (*in silico*, *in vitro*, and *in vivo*) can be taken. For example, on the whole, for prediction of human oral absorption, it appears that the rat is a good model and indeed the rat can be used successfully for the prediction of volume of distribution and several clearance processes, especially passive processes. When active processes contribute to clearance, species differences complicate extrapolation to human and predictivity is not at the level required. Finally, drug–food and drug–drug interactions are complicated by physiology and species differences and preclinical *in vivo* experiments are not recommended.

As drug metabolism science evolves and the human proteins modulating drug disposition are discovered and evaluated, there will be the opportunity to replace animal use with scalable *in vitro* assays. As this continues, drug metabolism scientists need to continually reassess the appropriate use of *in vivo* models of drug metabolism.

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