ADME/PK as part of a rational approach to drug discovery

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Rational drug discovery requires an early appraisal of all factors impacting on the likely success of a drug candidate in the subsequent preclinical, clinical and commercial phases of drug development. The study of absorption, distribution, metabolism, excretion and pharmacokinetics (ADME/PK) has developed into a relatively mature discipline in drug discovery through the application of well-established *in vitro* and *in vivo* methodologies. The availability of improved analytical and automation technologies has dramatically increased our ability to dissect out the fundamentals of ADME/PK through the development of increasingly powerful *in silico* methods. This is fuelling a shift away from the traditional, empirical nature of ADME/PK towards a more rational, *in cerebro* approach to drug design.

Much attention has been focussed on the demands that combinatorial chemistry and HTS are placing on the beleaguered ranks of drug metabolism scientists, requiring them to seek evermore ingenious approaches to deal with smaller and smaller quantities of increasing numbers of compounds. However, in the headlong rush for new technologies, it is important not to lose sight of the goal, namely the desire to 'front-load'[1] the drug discovery process to improve the likelihood of successfully negotiating the later, more expensive stages of drug development. For ADME/PK, this strategic shift has been occurring for some time, driven by concerns that poor pharmacokinetics was the major cause of compound failure in drug development programmes, as suggested by Prentis *et al.* in the mid-1980s (Ref. 2). ('Poor' pharmacokinetics is a somewhat subjective term, as the absolute requirements will depend on the product profile. However, it would typically include such things as low and variable bioavailability and insufficient systemic exposure.) Considering pharmacokinetics to be the weakest link in the drug development chain was a major influence in expanding ADME/PK from its traditional role as a preclinical safety support function towards the earlier stages of drug discovery. Moreover, this shift was relatively easily accomplished using existing methodologies. However, the establishment of a comprehensive front-loaded drug discovery programme is now hampered both by practical considerations, such as the small quantities of many (probably impure) compounds available for testing; and, more fundamentally, by the complexities of physiology, which in many areas, particularly toxicology, continue to frustrate attempts to develop valid *in vitro* methods. However, it is worth noting that current limitations in obtaining meaningful information on properties such as pharmacokinetics and toxicity are often eclipsed by an inability to anticipate the vagaries of the commercial arena to which the products are targeted.

Our own appraisal of the causes underlying compound failure in development, based on analysis of in-house data and a re-evaluation of the published work mentioned earlier[2], suggests that no single factor, such as poor pharmacokinetics or toxicity, can be highlighted as the major cause. Rather, a relatively even spread of issues underlies the current attrition rate typical of the pharmaceutical industry. Moreover, the assignment of failure to a distinct category might itself be misleading, as factors such as efficacy, toxicity, solubility and pharmacokinetics are all inter-related. For instance, toxicity might be cited as the ultimate reason for termination of a compound in development, but the pertinent factors might actually be

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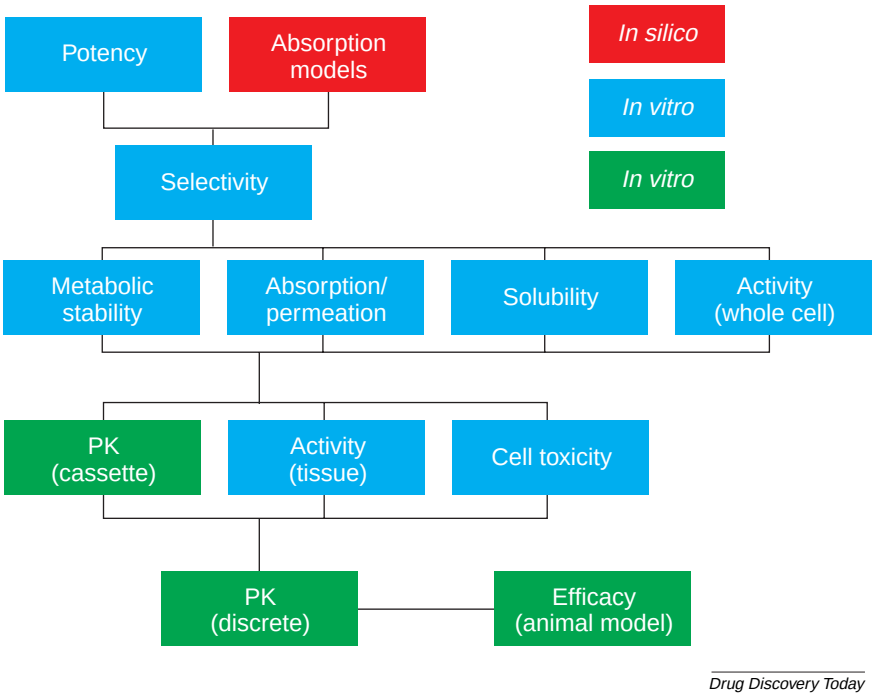

Figure 1. *A typical 'parallel' progression strategy for lead optimization. Abbreviation: PK, pharmacokinetics.*

prolonged and unnecessary systemic exposure (i.e. poor pharmacokinetics) or the need to administer high doses because of low potency (i.e. poor efficacy).

Clearly the development of molecules that have the greatest likelihood of commercial success requires a combined evaluation of all the contributing factors during the lead optimization phase. Therefore, while this article deals only with the role of ADME/PK studies during drug discovery, it is important to remain aware of these interrelationships during compound progression.

Defining an ADME/PK strategy

Four factors are primarily responsible for determining the nature and extent of ADME/PK studies within lead optimization. These are the phase of the discovery programme, the number of compounds available for study, the tools available and, most importantly, the specific requirements for a drug candidate defined within the product profile.

ADME considerations prior to lead optimization

Having become established as a routine part of lead optimization, increasing attention has been given to the role of ADME/PK at the earlier stages of discovery. The target identification and hits-to-leads areas have traditionally been something of an ADME/PK-free zone, as both the desire for such data and the means of generating them on a large scale have been lacking. The development of assays for cytochrome P450 inhibition, which use fluorimetric[3] or radiometric[4] endpoints, now offers the opportunity to screen for this property alongside primary assays for activity. However, it is still questionable from a project perspective whether the value of having this information at such an early stage would warrant the considerable cost of generating it. The number of compounds requiring ADME/PK evaluation at this stage is often small, comprising either potential leads, competitor compounds or biochemical tools. Consequently, drug metabolism scientists can influence the early stages of drug discovery without heavy reliance on high-throughput screens. Indeed, characterization of the *in vivo* pharmacokinetics of competitor compounds and potential tools can be extremely valuable during target validation to ensure that lack of appropriate systemic exposure does not undermine the effectiveness of a disease model. Information on pharmacokinetic–pharmacodynamic relationships can also be useful in defining product profiles.

The assessment of template tractability through the study of potential leads is a more fundamental principle of rational drug discovery. The path of lead optimization is largely determined by the starting template, which has traditionally been selected entirely on pharmacological activity and pharmacophore information. Although these still remain the key properties, the subsequent process can be streamlined by a much earlier assessment of the other factors, such as safety and pharmacokinetics, which are important in developing medicines that realize clinical and commercial viability. While mindful of the risk of inappropriate rejection of possible leads, selection of the most attractive starting point should hopefully minimize the number of iterations required to produce a suitable drug candidate. At the very least, having this additional information early provides an awareness of any shortcomings that will need to be addressed during the optimization process. Furthermore, as the ability to predict ADME in humans on the basis of physicochemical and basic *in vitro*

data improves, it should become possible to eliminate those apparent leads that, in reality, prove impossible or impractical to refine, and currently waste many valuable resources.

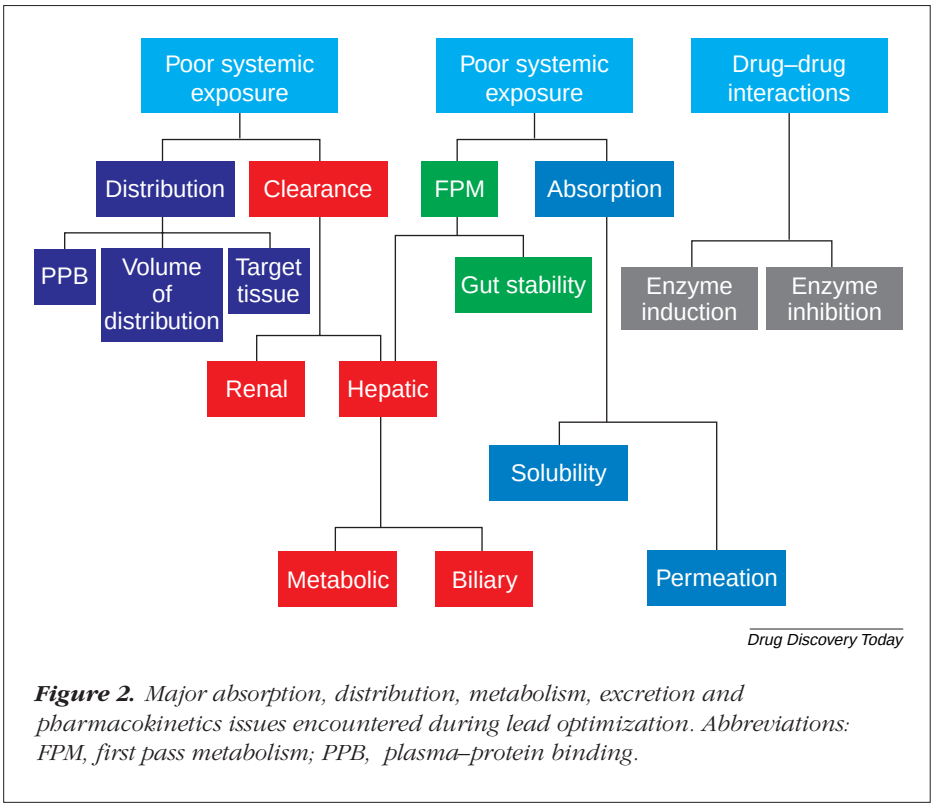

Figure 2. *Major absorption, distribution, metabolism, excretion and pharmacokinetics issues encountered during lead optimization. Abbreviations: FPM, first pass metabolism; PPB, plasma–protein binding.*

Streamlining rational lead optimization

After a suitable lead compound has been identified, the task of optimizing this template begins in earnest. The traditional linear process that began with improving potency to the exclusion of all else has recently been replaced in enlightened groups by a more parallel approach, such as that shown in Fig. 1 (Refs 5,6), which takes account of the need to build in drug-like properties simultaneously. In taking this approach, the key issues for ADME/PK studies are 'relevance' and 'capacity'.

The increased speed with which medicinal chemists and biologists can synthesize and test new molecules has caused a shortening of the iteration time of a drug discovery project. Drug metabolism scientists must provide pharmacokinetic and metabolic information within a similar timeframe in order to maintain influence over project progression. With finite resources, it becomes even more important to ensure that the studies carried out address only those issues relevant to the specific project.

The common ADME/PK issues encountered during lead optimization (Fig. 2) are concerned essentially with systemic exposure, although an awareness of the potential for drug–drug interactions[7] (The Food and Drug Investigation guidelines concerning the implications and investigation of drug–drug interactions can be found at http://www.fda.gov/cder/guidance/2635fnl.htm) is increasing at this stage. Problems with systemic exposure typically became apparent through the failure of molecules with *in vitro* potency to show subsequent efficacy *in vivo*. Hence, it is now more common for initial ADME/PK studies to be conducted prior to *in vivo* efficacy testing to evaluate systemic exposure and make best use of animal models, whose use is often constrained for practical and ethical reasons. The capacity to relate systemic drug concentrations to a pharmacodynamic effect can ensure that rational decisions are made in the context of the target product profile. For instance, a project might strive excessively to meet targets for the elimination half-life of a product that would be consistent with once-a-day dosing, when in reality the duration of action is not directly governed by the plasma concentration[8,9]. While aiming for a long half-life is undoubtedly an advisable general principle of lead optimization, it is important to ensure that the time and effort expended are justified on the basis of likely improvements in the pharmacodynamic profile.

After establishing that ADME/PK findings are relevant to compound progression, it is necessary to investigate the underlying cause(s) of any problems that might be encountered. A good understanding of these problems is the cornerstone of a rational ADME/PK lead optimization strategy because it ensures that the appropriate tests are conducted and that the synthetic programme is directed accordingly[10]. An interesting example of this principle is described by Smith *et al.*[11] for a series of thromboxane synthetase inhibitors/thromboxane A_2 antagonists. Despite having differing metabolic labilities, the clearance of these compounds in the rat is similarly high and was subsequently found to be governed by active hepatic uptake. An approach aimed at reducing clearance by improving metabolic stability would thus have failed, unless it also happened fortuitously to remove the susceptibility to uptake.

Selecting the appropriate tools

Having identified the key ADME/PK issues, attention can be given to the means with which to investigate them. Here, capacity is usually the crucial factor, comprising both the number of compounds that can be processed routinely and the time taken for results to be generated. ADME/PK screening is usually taken to mean *in vitro* systems for studying absorption and metabolism. However, *in vivo* studies still provide the definitive assessment of overall drug disposition, and progress has been made in overcoming some of the constraints associated with this approach. Cassette dosing is now an established method within the pharmaceutical industry as it provides a relatively quick way of ranking compounds according to their pharmacokinetic properties and requires the use of fewer animals[12]. Automation of the pre- and post-in life stages of both cassette and discrete PK studies also reduces the time and effort required to conduct such work[13].

The relative simplicity of *in vitro* systems for studying ADME/PK can be both an advantage and a disadvantage. The absence or control of complicating factors such as blood flow, protein binding, pH and co-factor availability means that specific mechanistic issues can be studied in detail. However, this also means that absolute correlations between *in vitro* and *in vivo* behaviour are often difficult to establish routinely, particularly for humans[14–17]. For this reason, *in vitro* screens are best viewed as a means of ranking compounds for further study rather than for outright rejection. Moreover, it is important that *in vitro* screens are regularly validated against *in vivo* data to ensure that decisions based on *in vitro* data remain relatively sound, if not totally predictive, particularly as the chemical series evolve.

The most commonly used *in vitro* systems for ADME/PK screening are those used for assessing metabolic stability and enzymology[18,19], as well as permeation across membranes such as the gastrointestinal tract and the blood–brain barrier (BBB)[20–22]. An additional advantage of such *in vitro* systems is the availability of human-derived or human-like materials that can help to give a view of likely behaviour in the target species.

In vitro methods, by their nature, are generally amenable to miniaturization and automation, which provides improved capacity. Even the use of cell-based systems such as Caco-2 or Madin–Darby canine kidney models for measuring absorption and BBB penetration can be automated to a large extent[23]. With most *in vitro* systems, it is the analytical requirements that are usually rate limiting, relying heavily on liquid chromatography coupled with mass spectrometry or liquid chromatography–tandem mass spectrometry. The major exceptions to this are the fluorimetric and radiometric techniques mentioned earlier, but the lack of a truly generic assay for metabolic stability or absorption currently limits the capacity of such screens.

'Mass customization' of ADME/PK screens

It might seem that the twin requirements for relevance and capacity within ADME/PK screening already outlined are not easily reconciled. The idea of performing bespoke studies to meet the specific needs of individual discovery projects does not sit easily with the widespread development of high-throughput approaches based on the automation of standard protocols. In this respect, scientists involved in ADME/PK screening are facing a problem analogous to that currently confronting car makers and many other manufacturing industries, which have previously been reliant on mass production. The challenge for drug metabolism scientists is similarly to establish a process of 'mass customization', whereby technology platforms are developed that have sufficient flexibility to accommodate studies tailored for specific needs while still providing the efficiency gains necessary to meet project demands.

In silico *approaches*

Notwithstanding the development of faster and smarter screening technologies, the biggest factor in determining the future role of ADME/PK within drug discovery is the advent of *in silico* techniques for rationalising and predicting these properties. Practical approaches for studying ADME/PK still require that molecules are made and then tested. The empirical nature of this work means that its role in drug discovery is limited, particularly now that virtual libraries can be generated by computer and tested in models of receptors and enzymes or compared with pharmacophores of known actives[24]. This development needs to be matched by the generation and application of *in silico* ADME/PK routines, and this is where the increased pace of data generation and collection within drug metabolism has helped. Now an understanding of the principles relating chemical structure to ADME/PK behaviour can be based on data from thousands of molecules and not just the handful on which previous rationales have been based[25,26]. The application of informatics to ADME/PK can provide general guidance on properties such as absorption and BBB penetration[27,28] and such 'rules of thumb' have been the mainstay of ADME/PK scientists for some time. However, the combination of a wide ADME/PK database and modern computational chemistry techniques enables a more rigorous appraisal of the properties of a molecule as a whole, rather than just relying on knowledge of isolated fragments and functional groups. Furthermore, these new models have applicability across a much wider range of the chemical universe than was hitherto the case.

While there is still some way to go before the ADME/PK needs of drug discovery can be carried out entirely by computer, *in silico* methods are already finding utility in several aspects of the process. One model developed enables an accurate ranking of compounds according to their metabolic stability. This model is based on data generated from an automated screening system for determining stability in human liver microsomes[18] and can now be used as an efficient 'pre-screen' to limit the need for future high-capacity metabolism screening. This not only saves resources but means that capacity is available to screen sets of compounds selected for the primary purpose of exploring the structure–activity relationship of drug metabolism and drug absorption. In the same way, these models have also been used as an ADME/PK filter for compounds prior to further pharmacological testing, where the capacity for such testing was unable to cope with the number of hits in primary screening. The obvious next step from influencing the primary *in vitro* pharmacological screening is for *in silico* models to be used in assisting library design by virtual screening of proposed structures to ensure that ADME/PK properties are favourable before carrying out the synthesis.

Conclusions

Rational lead optimization requires an appraisal of ADME/PK issues alongside other 'developability' factors from the earliest stages of drug discovery. The deployment of available *in vivo* and *in vitro* methods must be based on a sound understanding of the relevant ADME/PK issues, as they affect the desired product profile, and not simply on the capacity to process large numbers of compounds. High-throughput techniques have dramatically increased the size and quality of the database from which to gain a perspective on factors such as metabolic stability, absorption and enzymology. Consequently, advances being made in understanding the principles underlying these processes through the development of *in silico* methods are enhancing the conceptual nature of ADME/PK, and might lead ultimately to a truly rational, *in cerebro* approach to drug design.

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