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MiniReview

Prediction of Drug Metabolism and Interactions on the Basis of *in vitro* Investigations

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Abstract: Drug metabolism profoundly affects drug action, because almost all drugs are metabolised in the body and thus their concentrations and elimination rates are dependent on metabolic activity. Drug metabolism contributes substantially to interindividual differences in drug response and is also often involved in drug interactions, resulting in either therapeutic failure or adverse effects. Knowledge about the metabolism of a new chemical entity and its affinity to drug-metabolising enzymes helps in the drug development process by providing important information for the selection of a lead compound from among a number of substances pharmacologically equally effective in their therapeutic response. In drug development protocols, metabolism characteristics should be assessed very early during the development process. This has been made possible by the advances made especially in analytical capabilities and in *in vitro* technologies that are employed to predict *in vivo* metabolite profile, pharmacokinetic parameters and drug-drug interaction potential.

The principal route of elimination of drugs from the body is enzymatic biotransformation. The oxidative reactions are mainly catalysed by cytochrome P450 (CYP) enzymes (phase I metabolism) (Rendic & DiCarlo 1997; Guengerich & Rendic 2002) and, after that, by conjugating enzymes (phase II metabolism). Especially glucuronidation, catalysed by the several UDP-glucuronosyltransferase isoforms is an important route of phase II drug metabolism in humans. Some prodrugs need to be metabolically activated before they are pharmacologically active. This activation usually occurs via hydrolytic enzymes, or in some cases, by CYP enzymes.

Many drug-metabolizing enzymes constitute extensive and complex families, even superfamilies, with many individual members exhibiting distinct, but often overlapping selectivities towards substrates and inhibitors. Furthermore, expression of most drug-metabolizing enzymes varies between individuals due to genetic, host and environmental factors and some diseases (Pacifici & Pelkonen 2001). These factors produce huge inter-individual variation in the rate and metabolic pathways of drugs. One example of genetic factors influencing the inter-individual and inter-population variation is the polymorphic expression of many P450 and glucuronosyl transferase enzymes in the population (Ingelman-Sundberg *et al.* 1999). The frequency of poor and ultrarapid metabolisers varies markedly between ethnic groups. Some external factors, such as dietary compounds, cigarette smoking, alcohol and drugs may cause induction or repression of the expression of certain P450s. However, *in vitro* systems for predicting induction are not covered here to any extent and a reader is referred to recent reviews (Pelkonen *et al.* 2002b; Honkakoski 2003).

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The need to assess metabolism in early drug development

Elucidation of metabolic stability, metabolic routes, metabolising enzymes and their kinetics, and metabolic interactions is important for pharmaceuticals, because this information is needed for selecting leads and candidate drugs during drug discovery and development. Furthermore, lack of detailed knowledge of the metabolic fate and interactions of marketed drugs can result in morbidity or mortality, therapeutic failure, and toxicity from unanticipated overdose or metabolic reactions. In short, metabolism determines to a great extent the pharmacokinetic properties of most drugs and is often behind bioavailability problems, interindividual variations, metabolic interactions, idiosyncrasies and so on (White 1998; Boobis *et al.* 1999; Pelkonen *et al.* 2002a). This fact is the overpowering rationale for *in vitro* methods to assess drug metabolism and metabolic interactions in conjunction with drug discovery and development.

What do we want to know about drug metabolism?

There are two important goals for early *in vitro* studies during drug development: to elucidate metabolic stability in

order to predict metabolic clearance of the compound, and to assess drug-drug interaction potential. There are a number of approaches to provide data for the prediction concerning both goals (see below). Other important, and partially overlapping, goals are the elucidation and determination of principal metabolic routes of a new chemical entity and the tentative identification of principal metabolites, the identification of the enzymes catalysing the principal biotransformation routes (primary and important secondary metabolites) and gaining some quantitative data on their significance for the overall metabolic fate of a new chemical entity; and the provision of useful background information for characterising physiological, genetic and pathological factors affecting the kinetics and variability of a new chemical entity in the *in vivo* situation. It is also of considerable importance to gain some insight into interspecies differences, especially between human and species used in toxicity testing, in order to estimate whether there are any metabolic problems in the extrapolation of findings from animal toxicology studies to the human situation. Especially, all metabolites found from human should also be found from toxicology animals to be certain that all factors contributing to toxicity were involved.

Analytical tools for measuring a parent compound and metabolites

The most important prerequisite for drug metabolism studies is the availability of methods to identify and quantificate both the parent compound and its principal metabolites (Nassar & Talaat 2004). Especially the metabolite identification was a very challenging task even a few years ago and a major impediment for the sufficient characterization of preclinical ADME of new chemical entities. With the advent of novel mass spectrometry (MS)-based techniques such as time-of-flight (TOF) mass spectrometry, has made the tentative identification of principal (and minor) metabolites produced from a new chemical entity a relatively straight-forward and quick task, at least at the level of identification of biotransformations. The superior whole mass range sensitivity of modern TOF-MS instruments makes it possible to detect and identify unpredicted and minor metabolites. The ability to easily confirm biotransformation by measuring exact masses of molecules, which are anticipated as possible metabolites of a molecule, makes possible the construction of comprehensive metabolic map at an early stage of investigations of a new chemical entity. An unequivocal identification of a metabolite needs, however, additional mass spectrometric techniques besides TOF-MS and usually even the production, perhaps by enzymatic means, and isolation of sufficient amount for NMR spectroscopy.

The development of a quantitative method for the measurement of the parent compound and its metabolites produced may need LC/MS/MS techniques (Kostiainen *et al.* 2003). Quantification with LC/TOF-MS is also possible and sufficient even though it has limited linear range and somewhat poorer sensitivity and selectivity compared to LC/MS/MS. Sample matrix in *in vitro* experiments is not at all as filled with endogeneous molecules as the matrix later in *in vivo* experiments, where LC/MS/MS is clearly the workhorse of quantitative analysis. On the other hand, quantification with LC/MS/MS is robust for high through-put analysis, which makes it ideal for large sample sets. At the moment, metabolite identification is not suitable for high through-put analysis, because intellectual expertise is needed during the identification process. However, every instrument vendor have add-in software to automatically produce and process LC/MS data and search for metabolites. These software surely help higher through-put metabolite identification, but a lot of expertise is needed from the operator to be able to produce useful and reliable metabolite data. In any case, novel LC/MS methods have improved and made quicker both the identification and measurement of metabolites, which means that at least semiquantitative metabolic map is available relatively early during drug development.

Enzyme sources for *in vitro* systems

Extrapolation of the results from animal studies to the human situation is usually difficult and contains many sources of errors. The most important reasons for this are the species-specific differences in drug-metabolising enzymes, both qualitative (different metabolic pathways) and quantitative (different intrinsic clearances), between the human being and the test species (Pelkonen & Breimer 1994). Therefore, "humanized" experimental systems early in drug development process are needed. There are currently several *in vitro* enzyme sources available for *in vitro* systems in connection with drug development: purified or recombinantly expressed enzymes, human liver microsomes or homogenates, liver slices, human hepatocytes and permanent cell lines either as such or containing single or multiple transfected human enzymes (Pelkonen *et al.* 1998; Plant 2004). A summary of the major biological preparations for *in vitro* studies is provided in table 1. Human liver samples obtained under proper ethical permission have been used as a golden standard. These preparations have usually been extensively characterised to be used for the primary screening (sufficient model activities, no known polymorphisms, expected effects of model inhibitors, quantitation of CYPs by Western blotting). A thorough treatment of these experimental systems can be found in recent review articles (Pelkonen *et al.* 1998, Taavitsainen *et al.* 2002, Kremers 2002; Donato & Castell 2003; Plant 2004).

Liver homogenate with appropriate co-factors can be used for metabolic stability studies when the biotransformation pathways of a new compound are unknown. Liver homogenate fortified with appropriate cofactors exhibits enzyme activities that are present in intact tissue. In incubations with liver homogenate, the whole spectrum of *in vitro* metabolites is formed and identification of metabolites of an unknown compound by the above mentioned

novel mass spectroscopy methods is possible. When the transport of a new chemical entity through cell membranes is under study, whole cell systems or liver slices are a good choice, since in these preparations the transport systems into and out of the cell are present, and even the cell-cell connections are preserved in liver slices. These preparations are also suitable for induction studies. Recombinant enzymes are used for the identification of enzymes metabolizing the compound under study, but they are also increasingly studied as an alternative method to predict hepatic clearance (Rodrigues 1999).

Which drug-metabolising enzymes should be covered?

A crucial goal in the characterisation of any biological preparation used in the *in vitro* test system is to delineate its spectrum and activity of drug-metabolizing enzymes. There are several important considerations related to this matter. First of all, drugs are metabolized by a plethora of enzymes in the body, each one by its specific and unique spectrum of enzymes complementary to its chemical structure. A list of enzymes catalysing the metabolism of at least some drugs is given in table 2. Although usually drugs are metabolised by the so called 'drug-metabolizing enzymes', some drugs are metabolised by enzymes, which are usually identified as important catalysts of endogenous pathways, such as monoamine oxidase. Consequently, it is impossible to be totally comprehensive with any *in vitro* system. Because complete comprehensiveness is impossible, the selection of enzymes to be covered in the first screening systems is dictated primarily on a statistical basis, i.e. which enzymes catalyse the biotransformation of the majority of drugs. On the other hand, it is be preferable to enhance the chances to cover as many potential drug-metabolizing enzymes as possible, for example by using human liver homogenate with the whole complement of appropriate cofactors in the primary metabolic stability assay instead of human liver microsomes. Obviously, when the chemical structure of the substance points to a possibility of metabolism by a 'rarer' enzyme, this possibility should be checked with appropriate experimental systems, if available.

One further point may be worth mentioning: individuality of each chemical substance to be screened. It is a common finding that even quite small changes in chemical structure might lead to a large change in metabolites formed or spectrum of enzymes catalysing the metabolism. Sometimes specific metabolic reactions are catalysed by one of the more 'exotic' enzymes (table 2), which are not usually covered by screening systems. In these cases metabolic peculiarities are probably uncovered only during later phases of investigations.

Determination of various aspects of metabolism in *in vitro* systems

During the development of a new chemical entity, at least the following investigations are usually required, the earlier the better (table 3):

– Measurement of the disappearance of a chemical in human liver preparations; "metabolic stability"

Table 1.

Comparison of *in vitro* enzyme sources used in preclinical research.

Enzyme sources	Availability	Advantages	Disadvantages
Microsomes (Kremers 1999)	Relatively good, from transplantations or commercial sources.	Contains important rate-limiting enzymes. Relatively inexpensive technique.	Contains only phase I enzymes and UGTs. Requires strictly specific substrates and inhibitors or antibodies for individual DMEs.
Liver homogenates (Kremers 1999)	Relatively good. Commercially available	Contains basically all hepatic enzymes.	Liver architecture lost.
cDNA-expressed individual CYPs (Rodrigues 1999)	Commercially available.	Can be utilised with HTS substrates. The role of individual CYPs in the metabolism can be easily studied.	The effect of only one enzyme at a time can be evaluated. Problems in extrapolation.
Primary hepatocytes (Guillouzo *et al.* 1995; Gomez-Lechon *et al.* 2003)	Difficult to obtain, relatively healthy tissue needed. Commercially available. Cryopreservation possible.	Contains the whole complement of DMEs cellularly integrated. The induction effect of an NCE can be studied.	Requires specific techniques and well established procedures. The levels of many DMEs decrease rapidly during cultivation.
Liver slices (Beamand *et al.* 1993)	Difficult to obtain, fresh tissue needed. Cryopreservation possible.	Contains the whole complement of DMEs and cell-cell connections. The induction effect of an NCE can be studied.	Requires specific techniques and well established procedures.
Immortalised cell lines	Available at request, not many adequately characterised cell lines exist.	Non-limited source of enzymes.	The expression of most DMEs is poor or absent if characterised at all.

For further reviews, see Brandon *et al.* (2003) and Plant (2004).

Table 2.

(Preferably human) drug-metabolizing enzymes, which should/could/might be included in the *in vitro* assays.

Enzyme classes/enzymes	(Types of) drugs metabolised (examples)	Sources available	Need of incorporation into *in vitro* systems
Cytochrome P450 enzymes (about 13 'drug-metabolizing' enzyme forms)	Practically 90 % of all drug substances	Hepatocytes most versatile and comprehensive; other tissues and cell types selectively	Incorporation of P450-competent enzyme source is necessary for most purposes
An example: CYP1A1	Few pharmaceutics, principally polycyclic aromatic hydrocarbons and other carcinogens	Recombinant enzyme Mainly extrahepatic expression (e.g. placenta from smokers)	Induction usually required for expression Incorporation not necessary routinely
An example: CYP3A4	A majority, >50%, of all clinically used drugs	Hepatocytes, recombinant	Incorporation necessary for comprehensive screening
Flavin-monooxygenases (FMO) (6 forms)	Compounds with secondary and tertiary amines or sulphydryl groups (chlorpromazine, desipramine, methimazole)	Hepatocytes most versatile and comprehensive	Incorporation comes automatically with hepatocytes Specific incorporation only in the special circumstances
Prostaglandin H synthase	PAH-diols, Aflatoxin B1, aromatic amines	Liver, kidney, bladder (microsomes)	Incorporation advisable for toxicity screening?
Alcohol/aldehyde dehydrogenases and oxidases	Various compounds with alcohol and aldehyde functions (ethanol)	Many tissues; cytosol	Some incorporation comes automatically with hepatocytes or hepatic homogenate Specific incorporation only in special circumstances
Monoamine oxidase	Selegiline, moclobemide	Many tissues; mitochondria	Incorporation advisable in specific situations
Esterases/hydrolases/peptidases	compounds with cleavable ester/amide bond (procaine, succinylcholine, lidocaine)	Many tissues including blood and blood cells	At least some activity present when liver preparation is incorporated into the test system
Reductases	Many substances with azo, nitro and carbonyl functions (chloramphenicol, naloxone) Importance not well characterised	Present in hepatocytes (cytosol, some in microsomes)	Activity needs special circumstances Need of incorporation not adequately defined
UDP-glucuronosyl transferases (UGT) Glucuronide conjugation	Most drugs with suitable O-, S- and N-functional groups (morphine, diazepam, paracetamol)	Hepatocytes most versatile and comprehensive (microsomes) Subcellular systems need UDPGA	Incorporation of UGT-competent enzymes source is advisable for most purposes
Sulfotransferases (SULT) Sulfate conjugation	Phenols, alcohols, aromatic amines (paracetamol, methyldopa)	Hepatocytes most versatile and comprehensive (cytosol) Subcellular systems need PAPS	Incorporation not adequately defined
GSH transferases (GST) Glutathione conjugation	Epoxides, arene oxides, nitro groups, hydroxylamines (ethacrynic acid)	Hepatocytes most versatile and comprehensive (cytosol, microsomes)	Incorporation not adequately defined
Acyl-CoA glycinetransferase amino acid conjugation	Acyl-CoA derivatives of carboxylic acids (salicylic acid)	Hepatocytes (mitochondria)	Incorporation advisable only in special cases
N-acetyltransferases (NAT) Acylation	Amines (sulphonamides, isoniazid, clonazepam, dapsone)	Hepatocytes (cytosol)	Incorporation advisable only in special cases
Methyl transferases Methylation	Catecholamines, phenols, amines (L-dopa, thiouracil)	Various tissues (cytosol)	Incorporation advisable only in special cases

– Identification of principal metabolites; "metabolite profile and proposed metabolic tree of a chemical"
– Development of an analytical "routine" method for metabolites
– Identification of enzymes catalysing metabolic routes with the aid of diagnostic inhibitors and antibodies, recombinant enzymes and correlation studies
– Determination of the potential new chemical entity has to inhibit drug-metabolising enzymes and cause drug-drug interactions
– Enzyme kinetic characterisation of principal metabolic reactions; for scaling up and predicting *in vivo* kinetics of a new chemical entity

Metabolic stability of a new chemical entity (Obach *et al.*

1997): The metabolic stability of a new chemical entity determines, to a great extent, its future as a drug candidate. If a new chemical entity is rapidly metabolised in human liver preparations, its bioavailability *in vivo* is most probably too low for it to be a drug candidate. This naturally depends on the administration route of the drug. By determining the time and concentration dependence of metabolite formation from a new chemical entity on the disappearance of the entity *in vitro* in an appropriate system, its metabolic fate and half-life *in vivo* can be predicted. Similar studies performed in human and test species give valuable information for the selection of test species for pharmacokinetic and toxicological *in vivo* studies.

Identification of metabolites and metabolic routes (Lee & Kerns 1999; Kostiainen *et al.* 2003): Metabolite identification can be developed from incubations with human liver preparations, homogenates or microsomes. For example, mass spectrometric methods employing LC as a separative tool have evolved into extremely sensitive and accurate techniques (see above). By these methods, it is possible to determine with high accuracy the exact molecular masses and metabolite structures. Sample preparation for mass spectroscopy studies is a critical step, since the available chemical information of a new chemical entity is often limited. The incubation conditions and the reaction-terminating reagent have to be chosen so as not to alter the parent compound or the metabolites chemically and to keep the recovery of the substrate and the metabolites close to 100%. Otherwise, it is impossible to predict the pathways for biotransformation.

Identification of CYPs metabolising a new chemical entity (Pelkonen *et al.* 1998): After characterising the metabolic stability and metabolic routes of a new chemical entity, the *in vivo* prediction requires clarification of the drug-metabolising enzymes that participate in the *in vitro* biotransformation of the entity. Additionally, the identification of drug-metabolising enzymes helps to determine the potential of other chemicals, inhibitory drugs, dietary compounds etc., to cause drug-drug interactions. After determining the initial velocity conditions and enzyme kinetic parameters, CYPs involved in the metabolism of a new chemical entity can be characterised by chemical and antibody inhibitors selective or specific for respective CYPs. In case the *in vivo* concentrations are known, it is recommendable to use substrate concentrations close to the therapeutic level, if at all feasible.

Some typical enzyme identification experiments and their outcomes are the following:

* Effects of various diagnostic CYP-selective inhibitors on new chemical entity metabolism: an estimation of the role of each CYP enzyme to contribute to the metabolism of a chemical. On the basis of this information, it is possible to perform predictive calculations to the *in vivo* behaviour of a chemical.
* Ability of recombinant enzymes to catalyse metabolite formation of a new chemical entity: gives a direct indication of the potential of each recombinant CYP to catalyse the reaction. However, it is still somewhat uncertain of whether the results from recombinant enzymes could be extrapolated to the whole microsomes and to the *in vivo* situation. There have been attempts to devise schemes to perform *in vitro – in vivo* –extrapolations on the basis of recombinant studies (Rodrigues 1999; Donato & Castell 2003; Masimirembwa *et al.* 2003).
* Correlation of CYP-selective model activities with metabolite formation of a new chemical entity: gives an indirect indication of the participation of each CYP enzyme to catalyse the reaction. The results are, however, confirmatory and should be evaluated in the context of other approaches.

Table 3.

In vitro studies for the characterization of metabolism and metabolic interactions of potential drugs.

In vitro test	Preparations	Parameters	Extrapolations
Metabolic stability	Microsomes Homogenates Cells Slices	Disappearance of the parent molecule or appearance of (main) metabolites	Intrinsic clearance Interindividual variability
Metabolite identification and quantitation	Same as above	Tentative identification by (e.g.) LC/TOF-MS or radiolabelled compounds	Metabolic routes Semiquantitative metabolic chart
Identification of metabolizing enzymes	Microsomes with inhibitors or inhibitory antibodies Recombinant individual enzymes Hepatocytes	Assignment and relative ability of enzymes to metabolize a compound	Prediction of effects of various genetic, environmental and pathological factors Interindividual variability
Enzyme inhibition	Microsomes Recombinant enzymes Hepatocytes	Inhibition of model activities by a substance	Potential drug-drug interactions
Enzyme induction	Cells Slices Permanent cell lines (if available)	Induction of model activities (or mRNA)	Induction potential of a substance

For reviews, see articles in Boobis (1995); Boobis *et al.* (1999) and von Moltke *et al.* (1998). See also articles of Andersson *et al.* (2001); Pelkonen *et al.* (2001) and Salonen *et al.* (2003).

Table 4.

Useful *in vitro* probe substances for xenobiotic-metabolising human hepatic CYPs.

CYP	Selective substrates (reaction catalysed)	Remarks	Selective inhibitors	Remarks
1A2	Ethoxyresorufin (O-deethylation) Caffeine (N-demethylation) Phenacetin (O-deethylation) Melatonin (6-hydroxylation)	Inducible by cigarette smoking and polycyclic aromatic hydrocarbons	Furafylline Fluvoxsamine	High selectivity Low selectivity
2A6	Coumarin (7-hydroxylation)	Polymorphic	Tranylcypromine	Low selectivity
2B6	Bupropion (hydroxylation)	Inducible by phenobarbital-type inducers	Thio-Tepa Ticlopidine	High selectivity Low selectivity
2C8	Paclitaxel (6α-hydroxylation) Amodiaquine (oxidation)		Quercetin	Not selective
2C9	S-Warfarin (7-hydroxylation) Diclophenac (1-hydroxylation) tolbutamide (methylhydroxylation)	Polymorphic Inducible	Sulfaphenazole	High selectivity
2C19	Omeprazole (oxidation) S-mephenytoin (4-hydroxylation)	Polymorphic Inducible	fluconazole	Low selectivity
2D6	Dextromethorphan (O-demethylation)	Polymorphic	Quinidine	High selectivity
2E1	Chlorzoxazone (6-hydroxylation)	Inducible by ethanol	Pyridine disulfiram	Low selectivity Low selectivity
3A4	Midazolam (1′- and 4-hydroxylation) Testosterone (6β-hydroxylation)	Inducible by phenobarbital-like inducers	ketoconazole itraconazole	High selectivity (itra>keto)

For reviews, see Rendic and DiCarlo 1997; Pelkonen *et al.* 1998; Nebert and Russell 2002.

The inhibitory effects of a new chemical entity on drug-metabolising CYPs (Pelkonen *et al.* 1998; Kremers 2002; Tucker *et al.* 2001): The effects of the studied compound on metabolite formation in the selected model system, usually human liver microsomes, are evaluated by incubating the substrate and the studied compound and observing the metabolite formation of the specific substrate. From the therapeutic point of view, it is important to know which drug-metabolising enzymes the substance under development has affinity to. The effect of a new chemical entity on CYP-specific activities is studied by co-incubating series of dilutions of the study substance with a reaction mixture and a specific substrate. The effect of a new chemical entity is described as the concentration of the studied compound causing 50% inhibition of the CYP selective activities. If there is affinity towards some CYP-enzyme, the apparent K_i can be determined. By comparing the effects of a new chemical entity on the CYP specific-activities to the respective effects of diagnostic inhibitors, a tentative prediction of the *in vivo* situation can be made. However, it is worth stressing here that IC_{50} values for competitive inhibitors are dependent on the substrate concentration and consequently mode of inhibition should be studied.

After the above studies, it is possible to plan further preclinical, molecular, toxicological and clinicopharmacological studies, with focussed consideration of those CYP enzymes which are of importance for the metabolism and kinetics of a new chemical entity.

In vitro – in vivo scaling

In general, *in vitro – in vivo* scaling consists of two approaches: prediction of the intrinsic clearance (Cl_{int}) of a new chemical entity and prediction of drug-drug interactions. In the literature, methods for both purposes are extensively presented and discussed in the light of actual experimental or clinical studies (Houston & Carlile 1997; Obach *et al.* 1997; Ito *et al.* 1998; Houston & Galetin 2003).

It has to be stressed that these extrapolation contain a number of uncertainties, which have to be acknowledged. Other pharmacokinetic parameters and characteristics, such as plasma protein binding, the differential binding to various tissues, volume of distribution, and extrahepatic routes of clearance, should also be considered for the estimation of *in vivo* kinetics on the basis of *in vitro* studies (Lin 1998; Theil *et al.* 2003). Also, several other processes, concerning for example absorption and excretion, may be rate-limiting steps in the overall pharmacokinetic behaviour of a compound and thus extrapolations based only on metabolic characteristics may be totally misleading.

Whatever the exact value of inhibition constant (IC_{50} or K_i) measured in *in vitro* experiments is, it does not directly tell us that inhibition will be observed during the *in vivo* use of a compound. The critical factor in the term $[I]/K_i$ is the concentration of an inhibitor [I], which ideally means the concentration at the active site or a modulatory site. Obviously, this particular concentration is not known and surrogate values are usually used, such as total or free concentration in the plasma. Most authors think that the unbound, (i.e. free concentration) is the most appropriate to use, because it is only free drug that is able to transfer to hepatocytes and to the vicinity of CYP enzymes. However, it is conceivable – and for some drugs even shown – that many lipid-soluble drugs are concentrated in hepatocytes and consequently the actual concentration in the liver far exceeds that in plasma. Even the measurement of the partition between liver and plasma does not necessarily indicate the available portion of a drug to an enzyme, because a drug may be very tightly bound inside hepatocytes and may not be available to the active site of the enzyme. A recent comprehensive analysis based on 193 *in vivo* drug-drug interaction studies involving inhibition of CYP3A4, CYP2D6 and CYP2C9 (Ito *et al.* 2004) came to the conclusion that the use of total (i.e. not free) hepatic input concentration of inhibitor together with *in vitro* K_i values was the most successful method for the categorization of putative CYP inhibitors. A detailed and extensive treatment of modelling and predicting interactions of drug metabolism, including factors affecting partition between liver and plasma, can be found in Leemann & Dayer (1995).

Strategies for *in vitro – in vivo* extrapolation of parameters describing drug clearance and inhibition interaction potential have mainly been validated with rat data. In the rat, Michaelis-Menten kinetics is common. Extension to the human where CYP3A enzymes dominate brings more complexities, due to the prevalence of non-Michaelis-Menten kinetics. The question of whether these complexities are strongly evident *in vivo* remains unanswered. The problem of equating *in vitro* with *in vivo* kinetics for CYP3A substrates remains a challenge that needs to be further addressed (Houston & Galetin 2003).

None of the *in vitro* systems for drug metabolism is yet validated, but for example ECVAM (European Centre for the Validation of Alternative Methods) has long-term plans to perform some validation (Hartung *et al.* 2003). It is sufficient to point out that the necessary prerequisite for (pre)-validation is the availability of high-quality data base of *in vivo* pharmacokinetic parameters of a large number of drugs. Comprehensive quality-controlled or quality-checked data bases are not available or they include only a modest number of drugs. The lack of reliable data bases is also a major impediment for proper development and validation of *in silico* modelling and simulation approaches (van de Waterbeemd & Gifford 2003).

High-throughput screening in drug metabolism

The technology of higher-throughput absorption, distribution, metabolism and excretion screening is developing fast, but still the fastest ADME methods do not come within two orders of magnitude of the rates routinely achieved in drug industry by truly high-throughput discovery screening, e.g. in receptor-ligand interaction assays. Protocol simplification and the use of microtiter plate formats have made it possible for many metabolism assays to be automated, using robotic systems. Metabolism studies most amenable to high throughput screening include metabolic stability (intrinsic clearance) and drug interaction (CYP inhibition) assays (Li 2001; White 2000).

Conclusions

Metabolism is a major determinant governing both pharmacokinetics and clinical response (pharmacodynamics) of the majority of small molecule drugs and a great deal of effort is now directed at assessing key metabolic parameters at early stages of drug development. Several *in silico* and *in vitro* methods are now available for determination of metabolic features, often yielding data that reasonably well predict *in vivo* behaviour of the studied drug molecules. Further refining of these methods will provide us with methods having increasing precision and speed, allowing for truly high throughput analysis of metabolic features. In addition, analogous methodology will be employed on predicting absorption, organ uptake and efflux mediated by various transporter systems, plasma protein binding, and cellular determinants of intrinsic clearance. As an outcome of this development, it is likely that we will witness a diminishing number of drug candidates being withdrawn from clinical studies (or the market) due to major kinetic problems, such as strong metabolism interaction potential.

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