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Lead profiling

'In silico' simulations to assess the 'in vivo' consequences of 'in vitro' metabolic drug–drug interactions

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Recently, metabolic drug–drug interactions (M-DDI) have raised some high-profile problems in drug development resulting in restricted use, withdrawal or non-approval by regulatory agencies. The use of *in vitro* technologies to evaluate the potential for M-DDI has become routine in the drug development process. Nevertheless, in the absence of an integrated approach, their interpretation and value remains the subject of debate, and the vital distinction between a useful “simulation” and a precise “prediction” is not often appreciated. Various *in silico* softwares are now available for the simulation of M-DDI. However, a concerted effort by the industry is necessary to evaluate their use. The FDA has recently emphasised the importance of such collaboration to improve the crucial path to development of new drugs. *In silico* simulation of M-DDI has the potential to add significant value to this process.

Introduction

There have been several high-profile issues in drug development recently relating to problems with metabolic drug–drug interactions (M-DDI) (e.g. with terfenadine, fenfluramine, mibefradil, bromfenac, astemizole and cisapride). The con-

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The propensity of a drug to undergo clinically relevant interactions with concomitant medications can decide on commercial success or failure and in the extreme case even lead to withdrawal of the product from the market. Accurate early prediction of metabolic drug–drug interactions (M-DDI) is therefore a cornerstone of successful drug discovery and development. Amin Rostami-Hodjegan and Geoff Tucker have a long-standing track record in exploring the scientific background of metabolic drug–drug interactions. Their efforts have culminated in the development of the M-DDI prediction software SIMCYP. Here, they review the underlying science of the prediction tools currently available.

sequences have ranged from restricted use or withdrawal to non-approval by regulatory agencies [see the US Food and Drug Administration (FDA) site (<http://www.fda.gov/medwatch/safety.htm>) for an updated list].

Recently, there has also been an increased interest in programs and databases that may help to assess the likelihood of M-DDIs by identifying sources of relevant *in vitro* data and by facilitating access to information on reported interactions. The use of such information, together with the application of predictive models, may expedite the clinical prevention of M-DDIs as well as new drug development.

Although the use of *in vitro* methods to evaluate the potential for M-DDI has become routine in the drug development process, their interpretation and value remain the

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Glossary

AUC: area under concentration–time curve (amount × time/volume).

CL_{int}: intrinsic clearance.

CL_R: renal clearance.

CYP: cytochrome P450, a group of enzymes responsible for the metabolism of many xenobiotics.

E_{H,m}: fraction of drug passing through liver metabolised to metabolite of interest (*m*).

fe: fraction of drug clearance by renal excretion.

fm: fraction of drug clearance by metabolic route of interest (*m*).

fu_{mic}: fraction of drug unbound in microsomal preparations.

F_H: fraction of drug passing through liver not metabolised.

IC₅₀: concentration of inhibitor reducing intrinsic clearance by 1/2.

Ki: inhibition constant.

subject of debate within the pharmaceutical industry. Part of this controversy relates to the level of confidence in extrapolating from *in vitro* data to *in vivo* outcome (IVIVE) [1,2]. In this context, it is vital to appreciate the difference between a useful “simulation” and a precise “prediction”.

Simulation versus prediction

Simulation is but a first step on the road to prediction. In the absence of complete information, *in silico* IVIVE represents a simulation. Nevertheless, it is valuable in summarising the probable impact of all previous information, in posing “what

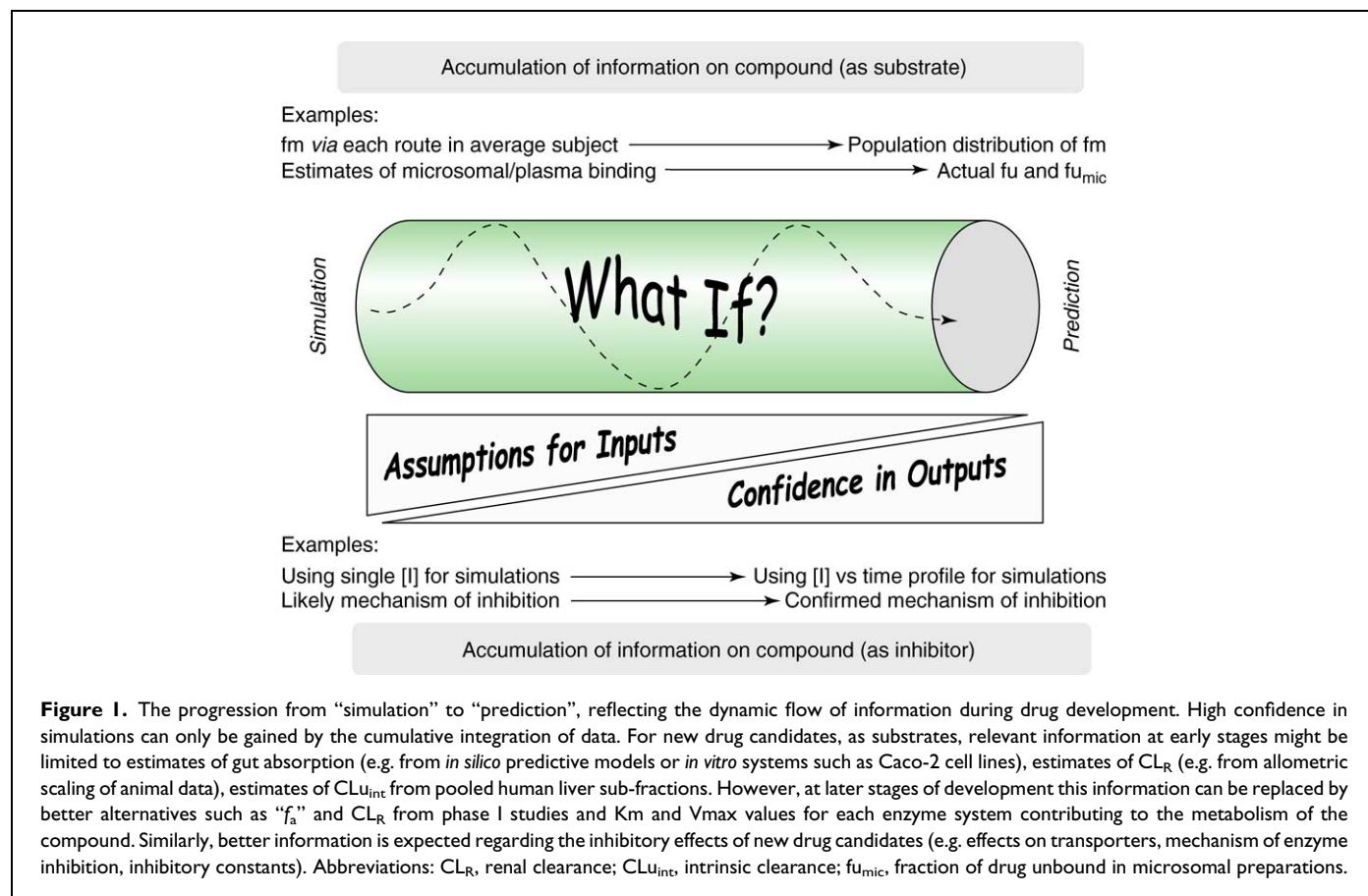
if” questions, in weighing the importance of missing data and in designing the next real experiment. Once further information becomes available, the simulation moves to becoming a prediction (Fig. 1). The ability to predict M-DDI accurately using IVIVE depends on the use of appropriate models and the availability of high-quality values for “all” model parameters from the *in vitro* data. Accordingly, many attempts at IVIVE, particularly those reported by the industry [3], lack sufficient key pieces of prior information or ignore obvious deficiencies in the data, resulting in unjustified claims that the process is not sufficiently ‘quantitative’ [1,2].

Methods and assumptions

Most approaches to calculate the level of a M-DDI rely on the following equation, describing the average increase in the area under the plasma concentration–time curve (AUC; see Glossary for definition of abbreviations) of a ‘victim’ drug following administration of a ‘perpetrator’ drug (after Rowland and Matin [4]):

$$\frac{\text{AUC (inhibited)}}{\text{AUC (uninhibited)}} = \frac{1}{\sum_{j=1}^n \text{fm}_j / \text{fold reduction in CL}_{\text{int},j} + \left(1 - \sum_{j=1}^n \text{fm}_j\right)}$$

where fm_{*j*} is the fraction of substrate clearance mediated by the inhibited metabolic pathway “*j*” and CL_{int,*j*} is the



intrinsic metabolic clearance of substrate down pathway j . The fold reduction in this value is defined according to the type of inhibition that occurs. Thus, for multiple (“ p ”) competitive inhibitors acting via same mechanism to inhibit enzyme “ j ”:

$$\text{Fold reduction in } \text{CLu}_{\text{int},j} = 1 + \sum_{k=1}^p \frac{[I_k]}{K_{i_k}} \quad (2)$$

where $[I_k]$ is the concentration of inhibitor “ k ” at the enzyme site and K_{i_k} the inhibition constant for inhibitor “ k ” obtained from *in vitro* studies after accounting for non-specific binding. The same equation applies for multiple non-competitive inhibitors acting at the same enzyme site. However, if the mechanisms of inhibition by multiple inhibitors are different (independent), the fold reduction in clearance would be greater:

$$\text{Fold reduction in } \text{CLu}_{\text{int},j} = \prod_{k=1}^p \left(1 + \frac{[I_k]}{K_{i_k}} \right) \quad (3)$$

In the case of mechanism-based (suicidal) inactivation of enzymes:

$$\text{Fold reduction in } \text{CLu}_{\text{int},j} = \frac{k_{\text{deg}} + ([I]k_{\text{inact}}/([I] + K_I))}{k_{\text{deg}}} \quad (4)$$

where k_{deg} is the natural degradation rate constant for the enzyme, k_{inact} the maximum degradation rate constant in the presence of a high concentration of inhibitor and K_I the concentration of inhibitor associated with half maximal inactivation.

Eq. (1) applies to orally administered drugs undergoing linear “first-pass” and “systemic” hepatic metabolism according to the “well-stirred” model. It ignores the possibilities of inhibition of “first-pass” metabolism in the gut wall, transient plasma binding displacement during the absorption phase and its effect on hepatic “first-pass” metabolism (which cannot be compensated by redistribution phenomena) and time-variant inhibitor concentration (leading to a different extent of inhibition of substrate metabolism during “first” and subsequent passes through the liver). Despite its relative simplicity, the model embodied by Eq. (1) requires a lot of prior information, including the concentration of inhibitor at the enzyme site, the mechanism of inhibition, inhibition constants for each route as well as proportional clearance of substrate via different routes (including renal excretion). These parameters are rarely characterised completely during early drug discovery and may not be available even during early drug development. For this reason, further simplifications are usually made, such as the assumption that the inhibited metabolic pathway is the only elimination pathway (i.e. $f_m = 1$), and that the inhibition is purely competitive. Hence, Eq. (1) becomes:

$$\frac{\text{AUC (inhibited)}}{\text{AUC (uninhibited)}} = 1 + \frac{[I]}{K_i} \quad (5)$$

This equation has been used for “semi-qualitative” predictions of M-DDI using a large database of compounds [5].

The ideal model for “predicting” M-DDI should include reliable values for all of the terms in Eq. (1) as well as a consideration of plasma binding displacement interactions and the impact of the concentration–time profile of the inhibitor. The latter feature is of particular importance when the design of the *in vivo* study being simulated involves single dose administration of both, inhibitor and substrate rather than multiple dosage of inhibitor before and following substrate dosage. Levels of enzyme inhibition more or less than expected from Eq. (1) are both possible, depending on the relative magnitude of plasma binding displacement of inhibitor by substrate drug and of substrate by inhibitor.

Inhibitor concentration at the enzyme active site

The “free drug hypothesis” indicates that the concentration of inhibitor used in the above equations should be the unbound concentration in liver water, assumed to be in equilibrium with the unbound concentration in plasma water. However, under-predictions of M-DDI on this basis have led many investigators to prefer the use of total plasma or even total liver inhibitor concentrations or maximum total inlet concentration to the liver [6]. But such underprediction could be for many other reasons, including lack of correction of K_i values for non-specific binding, underestimation of f_m owing to inadequate characterisation of metabolic pathways, concentrative transport of inhibitor into liver water, the generation of metabolites from inhibitor that also contribute to enzyme inhibition, neglect of inhibition of gut “first-pass” metabolism, and failure to detect mechanism-based inactivation of enzymes by appropriate *in vitro* studies.

Impact of non-specific binding on estimates of K_i and IC_{50}

The need to correct enzyme kinetic parameters for non-specific binding to microsomal protein was pointed out over 40 years ago [7], and reinforced more recently, specifically with respect to measurement of K_i values [8,9].

Austin *et al.* [10] have developed a function to predict non-specific binding to microsomal protein based on physico-chemical properties:

$$f_{u_{\text{mic}}} = \frac{1}{C \times 10^{0.56 \log P/D - 1.41} + 1} \quad (6)$$

where $f_{u_{\text{mic}}}$ is the free fraction of compound in the microsomal incubation, $\log P/D$ refers to its octanol/buffer partition coefficient (weak base, $pK_a > 7.4$) or distribution coefficient (neutral or weak acid, $pK_a < 7.4$) and C is the microsomal protein concentration in milligrams per millilitre.

Fractional metabolism by different pathways (f_m)

During early drug discovery, estimates of metabolic stability and intrinsic metabolic clearance are usually based on mea-

measurements of metabolic rate at a single, sub-saturating concentration in either recombinantly expressed human CYPs or pooled human liver microsomes. However, the contribution of a particular enzyme to overall metabolic clearance may be over- or under-estimated by this practice if this route or other pathways are partially saturated at *in vivo* concentrations, particularly during “first-pass”. Moreover, in the absence of an *in vivo* mass balance study in humans, information on the renal clearance of a candidate drug might not be reliable. Poor correlations between observed and predicted clearance using routine (and incomplete) metabolic data generated during discovery or early drug development stages are not uncommon [11]. However, because the ability to predict the extent of an M-DDI is highly sensitive to estimates of f_m , this parameter must be well defined with regard to its dependence on substrate concentration and in relation to non-metabolic elimination pathways.

Active uptake of inhibitor and generation of inhibitory metabolites

The active uptake of an inhibitor will lead to higher concentrations in tissue water than those expected from the unbound concentrations in plasma water. This possibility is difficult to study, but indications can be derived from experiments with hepatocytes at different temperatures and in the presence of general, relatively non-specific uptake inhibitors.

Examples of inhibitors where their metabolites contribute to the net effect include fluoxetine [12] and itraconazole [13]. Neglect of this issue will lead to underprediction of the interaction.

Inhibition of gut “first-pass” metabolism

Because CYP3A constitutes the major CYP enzyme in the gut wall, it is reasonable to assume that, knowing intrinsic clearance per unit of enzyme (from *in vitro* studies) and the overall abundance of the enzyme in gut [14,15], it should be possible to predict net intrinsic clearance by the gut and hence the extent of its inhibition. However, two compounds with exactly the same intrinsic clearance in the gut wall may exhibit different extents of “first-pass” gut metabolism. This is because intrinsic clearance only refers to the potential for metabolism when there are no restrictions. Exposure to enzymes during transit through the gut wall also depends on their interplay with uptake and efflux transporters, passive membrane permeability and enterocytic blood flow. These factors can be accommodated by a model that defines the availability of drug across the gut wall (F_{Gut}) in terms of intrinsic metabolic clearance ($CL_{U_{int,Gut}}$), the free fraction of drug at the enzyme site ($f_{u_{Gut}}$) and a nominal blood flow (Q_{Gut}) [16]:

$$F_{Gut} = \frac{Q_{Gut}}{Q_{Gut} + f_{u_{Gut}} CL_{U_{int,Gut}}} \quad (7)$$

Q_{Gut} can be further decomposed into a permeability clearance (CL_{perm}) and enterocytic blood flow (Q_{ent}). For a highly permeable drug (e.g. midazolam), Q_{Gut} asymptotes to the value of Q_{ent} .

$$Q_{Gut} = \frac{CL_{perm} Q_{ent}}{CL_{perm} + Q_{ent}} \quad (8)$$

Furthermore, permeability clearance (CL_{perm}) could be estimated from *in vitro* data (e.g. permeability in Caco-2 cells), enterocellularity and the relative abundance of transporter in the cell system and the gut. In the presence of an inhibitor that only alters intrinsic gut metabolic clearance, Eq. (7) can be rewritten to include an estimate of the concentration of inhibitor in the enterocyte ($[I]_{gut}$) and its inhibitory constant for CYP3A (K_i):

$$F_{Gut} = \frac{Q_{Gut}}{Q_{Gut} + f_{u_{Gut}} [CL_{U_{int,Gut}} / (1 + ([I]_{gut} / K_i))]} \quad (9)$$

When the inhibitor is not co-administered with substrate the unbound concentration of inhibitor in the systemic circulation can be used as an estimate of $[I]_{gut}$. However, when inhibitor and substrate are co-administered the value of $[I]_{gut}$ may be approximated from the pre-hepatic absorption rate of inhibitor and an estimate of enterocytic blood flow (Q_{ent}), according to Eq. (10):

$$[I]_{gut} = \frac{f_a \times k_a(I) \times \text{Dose}(I)}{Q_{ent}} \quad (10)$$

where f_a is the fraction of the inhibitor dose that is absorbed into the gut wall and $k_a(I)$ and $\text{Dose}(I)$ are the absorption rate constant and dose of inhibitor, respectively. The above equation assumes that the inhibitor is not subject to major “first-pass” gut metabolism itself.

Variation of inhibitor concentration with time

In many cases, M-DDI are primarily the result of inhibition of “first-pass” metabolism, because the concentration of inhibitor is highest during its absorption. This is demonstrated by simulations showing the large impact that staggering the times of administration of inhibitor and substrate can have on the degree of an interaction [17]. Predictions of M-DDI with Eq. (1) do not account for different levels of inhibition during “first-pass” and systemic exposure.

These differences may be further amplified by failure of drug to equilibrate between plasma water and erythrocytes or incomplete plasma binding during the short time between drug entering the portal vein and reaching the liver.

Mechanism-based inactivation of enzymes

As illustrated by Ito *et al.* [5] in their recent systematic review of M-DDI prediction, the degree of inhibition by mechanism-based inhibitors will be greater than that predicted from simple $[I]/K_i$ estimates. Typically, kinetic parameters describ-

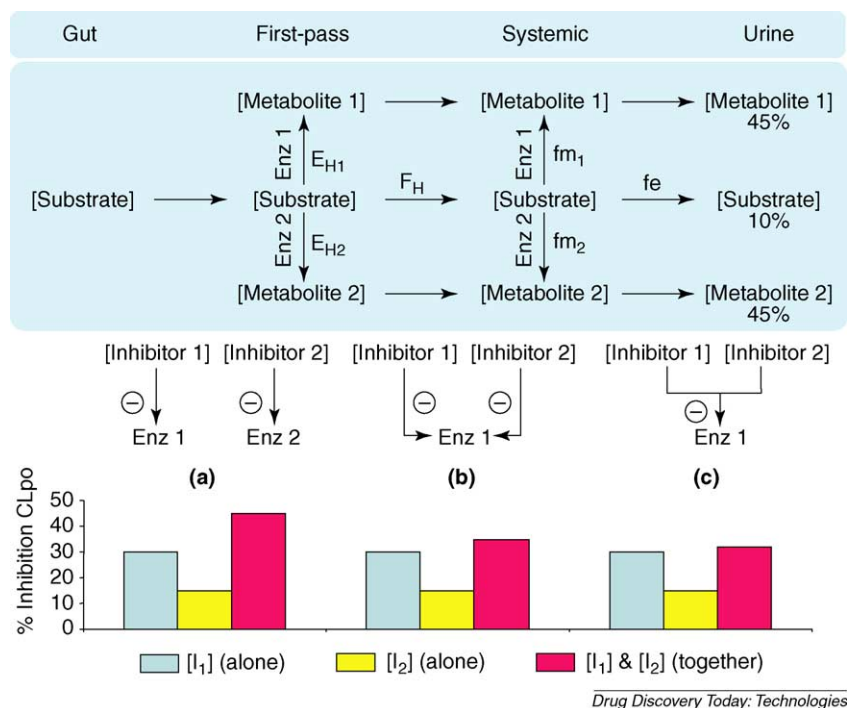


Figure 2. Three outcomes of multiple simultaneous M-DDIs, assuming no interaction between inhibitors I_1 and I_2 : (a) inhibition of different enzymes; (b) inhibition of the same enzyme by different mechanisms; (c) inhibition of the same enzyme by similar mechanism. The decrease in substrate clearance is additive only if the two inhibitors impair the activity of different enzymes [panel (a)]. Abbreviations: fe , fraction of drug clearance by renal excretion; F_H , fraction of drug passing through liver not metabolised; fm , fraction of drug clearance by metabolic route of interest.

ing this phenomenon are not always obtained as part of *in vitro* screening procedures, or the information is limited to a simple comparison of inhibition in pre-incubated samples (usually only at one or two time points) relative to those in which substrate and inhibitor are co-incubated.

Predicting the impact of multiple inhibitors

The worst, and probably the most clinically relevant, scenario involves administration of multiple inhibitors that block different metabolic pathways of the substrate compound. For example, if it is assumed that 10% of a victim drug is eliminated by renal excretion, 45% by metabolic route “ M_1 ” and 45% by metabolic route “ M_2 ”, and if inhibitor “ I_1 ” on its own can reduce the clearance by 30% by inhibition of the “ M_1 ” route, and “ I_2 ”, on its own, can reduce the clearance by 15% by inhibition of the “ M_2 ” route, then the overall decrease in clearance according to Eq. (1) would be additive (i.e. a 45% reduction in clearance = 30% by I_1 + 15% by I_2).

However, if the inhibitors both inhibit the “ M_1 ” route by independent mechanisms (e.g. I_1 is a mechanism based inhibitor and I_2 is a competitive inhibitor), the expected overall inhibition would be 35% (i.e. 30% owing to I_1 and 5% [15/45 of the remaining 15% of the “ M_1 ” route] by I_2).

A third scenario would involve inhibitors acting by the same mechanism and hence competing with each other for

net inhibition. Thus, although I_1 and I_2 might, for example, block 30% and 15%, respectively, of the metabolism when given on their own, when given together the net inhibition would only amount to 32% (Fig. 2).

Assessing the impact of population variability

Assimilating the complexities outlined above as well as incorporating other important issues, such as simultaneous inhibition and induction, requires a fully integrated approach to simulation. The value of the exercise is enhanced further if information on variability in enzyme function is included along with demographic, physiological, pathological and genetic variability, to produce “virtual populations”. For instance, consider a compound that is metabolised 50% by CYP1A2, 30% by CYP2D6 and that is excreted renally to the extent of 20%. Although complete inhibition of CYP1A2 in a typical healthy adult may not increase the AUC more than twofold, the same level of inhibition in an elderly patient with renal failure or an individual who lacks functional CYP2D6 for genetic reasons may cause up to 2.7- and 3.5-fold increases, respectively. The extent of inhibition in a poor CYP2D6 metaboliser with renal failure would be even greater (up to 11-fold assuming 25% normal renal function; Fig. 3).

Thus, an important aspect of *in silico* simulation should be an ability to assess and predict outcomes in populations

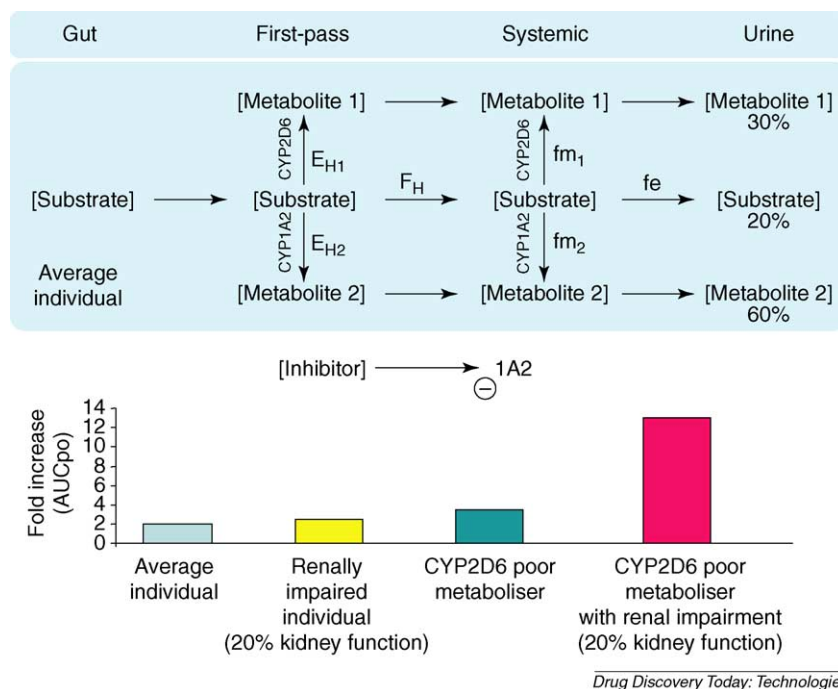


Figure 3. Amplification of the effect of primary inhibition of a particular enzyme (CYP1A2) by other attributes of an individual (renal impairment, CYP2D6 poor metaboliser genotype). Abbreviations: fe, fraction of drug clearance by renal excretion; F_H , fraction of drug passing through liver not metabolised; fm, fraction of drug clearance by metabolic route of interest.

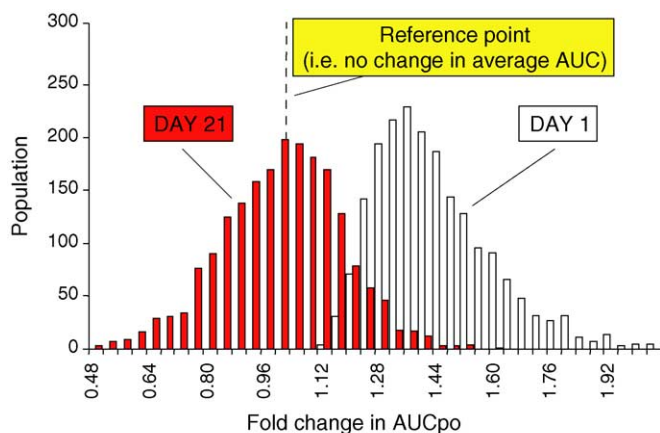
rather than in the non-existent ‘average’ patient. This is vital if individual patients at the extreme of risk are to be identified. Marked between-subject variation in the contribution of different enzyme isoforms underlines the need to study metabolic reactions in a sufficient number of livers when doing *in vitro* studies [18]. Furthermore, simulations of M-DDI should consider all conceivable combinations of individuals with different genetic make-up and renal function [19]. A clinical drug interaction study in 10 healthy volunteers (or even a larger number of patients) may provide inadequate assurance that a problem will not be

encountered when the drug is in wider patient use. By simulating a virtual population, incorporating a much broader range of demographic, physiological, genetic, enzymatic etc values, the exercise can provide early warning of the mix of patient characteristics predisposing to risk. Such individual features and their complex interplay are not always intuitively obvious.

Programs such as Q-DIPS [20] and databases such as that developed at the University of Washington [21] (<http://depts.washington.edu/didbase/>) go some way to accelerate obtaining information on “observed” M-DDIs (Table 1). They

Table 1. Examples of software/databases relevant to M-DDI

	University of Washington database	Q-DIPS	Simcyp [®]
Main purpose	A “mining” query system for retrieval of literature reports on <i>in vitro</i> and <i>in vitro</i> studies and their evaluation	A data collection system, initially conceived to be used by clinicians in assessing M-DDI	Simulation of <i>in vivo</i> consequences of <i>in vitro</i> data on drug metabolism obtained during drug discovery and development
Access to updated database	Online with regular update	Online access to bibliographic data	Selected database of probe substrates and inhibitors (updated bi-annually)
Access	Restricted to Members	Not clarified	Restricted to members
Training requirements	None	None	2-day workshops
Calculation of inhibition effects	No	Yes	Yes
Population-based prediction	No	No	Yes



Drug Discovery Today: Technologies

Figure 4. Simulation of the complex interaction between ritonavir and methadone involving simultaneous inhibition and induction. The extent of the increased plasma AUC (area under concentration–time curve) of methadone immediately after taking ritonavir will vary between different individuals. Moreover, the population distribution of the change in AUC shifts to the left with the duration of high dose ritonavir treatment as its inductive effect offsets its acute inhibitory effect.

help to flag out warnings to clinicians concerning interactions in the average individual, and direct investigators towards relevant studies. Q-DIPS is also able to simulate the magnitude of some M-DDI in an average individual. However, systematic algorithms that include population variability in the various components of models for M-DDI are required. In 2000, the University of Sheffield (<http://www.shef.ac.uk>) and several pharmaceutical companies formed a global consortium (<http://www.simcyp.com>) with the specific aim of producing automated platforms for IVIVE in virtual populations. The program (SIMCYP) incorporates extensive data on demographics, disease states, anatomical, physiological, genetic and biochemical variables, as well as input of information on *in vitro* drug metabolism and transport. The output of the simulations is presented in the form of population distributions of the extent of a M-DDI (Fig. 4). The project is ongoing and benefits from the advice provided by member companies as well as the FDA in updating databases and adding new features. It is also supported through one of the work-packages of a European Frame 6 grant (BioSim), which aims to structure efforts towards the development of *in silico* models for biological systems (e.g. virtual human populations) of relevance to drug design and evaluation.

Conclusions

Many large pharmaceutical companies are embracing the philosophy of using modelling and simulation technologies, and it has been suggested that *in silico* approaches may represent up to 15% of R&D spend in the next 5–10 years [22]. However, there are indications that implementation is not always optimal (see Outstanding issues for a list of some

outstanding issues). The reasons for this are many, and include excessive ‘compartmentalisation’ of departments (pre-clinical ADME does not always ‘talk’ to clinical PK-PD) and failure to acquire all of the necessary information at the right time in drug development (front-loading). Moreover, some of the tasks involved in building optimal *in silico* models require a joint effort on the part of several organisations, with implications for intellectual property (IP). However, as emphasised recently in the FDA’s white-paper on the crucial path to development of new drug products (<http://www.fda.gov/oc/initiatives/criticalpath/>; accessed in March 2004), without a concerted effort it is probable that many important opportunities will be missed. The use of *in silico* simulations to make better use of *in vitro* data during the development process could be one such opportunity.

Related articles

- Chien, J.Y. et al. (2003) Physiological approaches to the prediction of drug–drug interactions in study populations. *Curr. Drug Metab.* 4, 347–356
- Ito, K. et al. (1998) Prediction of pharmacokinetic alterations caused by drug–drug interactions: metabolic interaction in the liver. *Pharmacol. Rev.* 50, 387–411
- Tucker, G.T. (1992) The rational selection of drug–interaction studies – implications of recent advances in drug–metabolism. *Int. J. Clin. Pharmacol. Therap.* 30, 550–553
- Venkatakrishnan, K. et al. (2003) Drug metabolism and drug interactions: application and clinical value of *in vitro* models. *Curr. Drug Metab.* 4, 423–459
- Yao, C.P. and Levy, R.H. (2002) Inhibition-based metabolic drug–drug interactions: predictions from *in vitro* data. *J. Pharm. Sci.* 91, 1923–1935

Outstanding issues

- Better communication between large databases and predictive programs.
- Improved collaboration between companies in sharing databases on IVIVE cases.
- Rationalisation of IP issues related to anonymity and access to data and databases without compromising confidentiality.
- Commitment to dedicate personnel (realignment) to carry out prospective and retrospective evaluation of IVIVE.
- Redistribution of expenditure such that frontloading with high quality *in vitro* data becomes more common and the value of IVIVE is appreciated.

References

- Blanchard, N. *et al.* (2004) Qualitative and quantitative assessment of drug–drug interaction potential in man, based on K_i , IC_{50} and inhibitor concentration. *Curr. Drug Metab.* 5, 147–156
- Lin, J.H. (2000) Sense and nonsense in the prediction of drug–drug interactions. *Curr. Drug Metab.* 1, 305–331
- Rodrigues, A.D. *et al.* (2001) Use of *in vitro* drug metabolism data to evaluate metabolic drug–drug interactions in man: the need for quantitative databases. *J. Clin. Pharmacol.* 41, 368–373
- Rowland, M. and Matin, S.B. (1973) Kinetics of drug–drug interactions. *J. Pharmacokinet. Biopharm.* 1, 553–567
- Ito, K. *et al.* (2004) Database analyses for the prediction of *in vivo* drug–drug interactions from *in vitro* data. *Br. J. Clin. Pharmacol.* 57, 473–486
- Ito, K. *et al.* (1998) Quantitative prediction of *in vivo* drug clearance and drug interactions from *in vitro* data on metabolism, together with binding and transport. *Annu. Rev. Pharmacol. Toxicol.* 38, 461–499
- Gillette, J. (1963) Drug metabolism by enzyme mechanisms. *Prog. Drug Res.* 6, 55–57
- Tucker, G.T. (1992) The rational selection of drug–interaction studies – implications of recent advances in drug–metabolism. *Int. J. Clin. Pharmacol. Ther.* 30, 550–553
- Tucker, G. (1998) Extrapolating concentrations – *in vitro* to *in vivo*. In *European Cooperation in the Field of Scientific and Technical Research, European Symposium on the Prediction of Drug Metabolism in Man: Progress and Problems* (Vol. xiv) (Boobis, A.R. *et al.*, eds), p. 332, Office for the Official Publications of the European Communities
- Austin, R.P. *et al.* (2002) The influence of nonspecific microsomal binding on apparent intrinsic clearance, and its prediction from physicochemical properties. *Drug Metab. Dispos.* 30, 1497–1503
- Clarke, S.E. and Jeffrey, P. (2001) Utility of metabolic stability screening: comparison of *in vitro* and *in vivo* clearance. *Xenobiotica* 31, 591–598
- Crewe, H.K. *et al.* (1992) The effect of selective serotonin reuptake inhibitors on cytochrome-P4502d6 (Cyp2d6) activity in human liver-microsomes. *Br. J. Clin. Pharmacol.* 34, 262–265
- Isoherranen, N. *et al.* (2004) Role of itraconazole metabolites in CYP3A4 inhibition. *Drug Metab. Dispos.* 32, 1121–1131
- Paine, M.F. *et al.* (1997) Characterization of interintestinal and intrain-testinal variations in human CYP3A-dependent metabolism. *J. Pharmacol. Exp. Ther.* 283, 1552–1562
- Yang, J.S. *et al.* (2004) Cytochrome P450 3A expression and activity in the human small intestine. *Clin. Pharmacol. Ther.* 76, 391
- Rostami-Hodjegan, A. and Tucker, G.T. (2002) The effects of portal shunts on intestinal cytochrome P450 3A activity. *Hepatology* 35, 1549–1550
- Yang, J.S. *et al.* (2003) The effects of dose staggering on metabolic drug–drug interactions. *Eur. J. Pharm. Sci.* 20, 223–232
- Crewe, H.K. *et al.* (1997) Variable contribution of cytochromes P450 2D6, 2C9 and 3A4 to the 4-hydroxylation of tamoxifen by human liver microsomes. *Biochem. Pharmacol.* 53, 171–178
- Yang, J.S. *et al.* (2002) Prediction of ritonavir interaction with sildenafil (Viagra): incorporating population variability. *Br. J. Clin. Pharmacol.* 53, 438P–439P
- Bonnabry, P. *et al.* (2001) Predictive modelling of *in vivo* drug interaction from *in vitro* data: from theory to a computer-based workbench and its experimental validation. In *Interindividual Variability in Human Drug Metabolism* (Pacifci, G.M. and Pelkonen, O. eds), pp. 532, Taylor & Francis
- Carlson, S.P. *et al.* (2002) Development of a metabolic drug interaction database at the University of Washington. In Rodrigues, A.D. (ed.), *Drug–Drug Interactions* pp. 650, Marcel Dekker
- Uehling, M.D. (2003) *Model Patient*. *Bio-IT World* (<http://www.bio-itworld.com/archive/121503/trials.html>).