Pharmacokinetics in Early Drug Research

The Report and Recommendations of ECVAM Workshop 22

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Preface

This is the report of the twenty-second of a series of workshops organised by the European Centre for the Validation of Alternative Methods (ECVAM). ECVAM’s main goal, as defined in 1993 by its Scientific Advisory Committee, is to promote the scientific and regulatory acceptance of alternative methods which are of importance to the biosciences and which reduce, refine or replace the use of laboratory animals. One of the first priorities set by ECVAM was the implementation of procedures which would enable it to become well-informed about the state-of-the-art of non-animal test development and validation, and the potential for the possible incorporation of alternative tests into regulatory procedures. It was decided that this would be best achieved by the organisation of ECVAM workshops on specific topics, at which small groups of invited experts would review the current status of various types of in vitro tests and their potential uses, and make recommendations about the best ways forward.

The workshop on Pharmacokinetics in Early Drug Research was held in Bath, UK, on 27–29 March 1996, under the co-chairmanship of David Leahy (ZENECA Pharmaceuticals, Alderley Park, Macclesfield SK10 4TG, UK).
Pharmaceuticals, Macclesfield, UK) and Ruth Duncan (School of Pharmacy, London, UK). The aims of the workshop were to: a) review the current methodology for providing early pharmacokinetic and related information; b) define current best practice and identify those problem areas where scientific and technological advances could significantly improve the efficiency of drug discovery; and c) recommend further work which should be conducted in these high priority areas.

Introduction

The drug research and development process is evolving rapidly due to technological advances in the identification of biological targets, as well as in the automation of chemical synthesis and screening. The methodology used for designing compounds with good activities \textit{in vivo}, which is typically based on a combination of high-throughput synthesis and screening, will need to be adapted in the light of these technological advances. One challenge that presents itself is how to improve our efficiency in the optimisation of activity \textit{in vivo}, especially with respect to aspects of pharmacokinetics and safety assessment, without increasing our reliance upon animal testing.

The workshop was held to consider these changes, and to develop recommendations for priority areas of research that could lead to a more effective use of animal studies while increasing the capacity for efficient optimisation of activity \textit{in vivo}.

Lead Structure Identification

Drug discovery continues to undergo enormous changes, driven by technological innovation and the pressures of competition. Remarkable advances in the identification of new therapeutic targets have been seen, as a consequence of progress in molecular biology and the Human Genome Project. New chemical lead identification is becoming much easier as a result of two major developments: a) the revolution in synthetic chemistry due to the impact of robotics and combinatorial libraries; and b) the establishment of high-throughput screening procedures. It is probable that these changes will continue to be of great significance, as the Human Genome Project continues to deliver massive amounts of information, which will be analysed by increasingly sophisticated computer methods, and as the robotisation and miniaturisation of screening and synthesis procedures become more commonplace.

High-throughput primary screening can produce many thousands of different compounds which exhibit the required effects at relatively high concentrations. The identification of lead structures is the process by which these compounds are sifted (secondary screening) to provide congeneric series suitable for lead optimisation programmes. This requires a variety of rapid, relatively straightforward, relevant and reliable systems, which provide physicochemical and biological information.

Key physicochemical properties are solubility, stability, lipophilicity (as determined by measuring the octanol–water $P$ or membrane–water partition coefficients [2]) and pKa (the negative log of the acid dissociation constant); these can be used to predict protein binding, tissue distribution and gastrointestinal (GI) absorption [3].

There can be no standard approach to the use of biological models in the secondary screening stage, as this will depend upon the type of effect required, as well as on the number and variety of compounds identified in the primary screen. Estimations of the probability that compounds will reach adequate blood levels \textit{in vivo} for them to be active can be obtained from physiologically based pharmacokinetic (PBPK) models, or from relatively simple \textit{in vitro} experiments.

\textit{In vitro} cytotoxicity and genotoxicity tests are suitable for use with large numbers of compounds, and the use of freshly isolated hepatocytes in suspension [4], or microsomes, can provide important information on metabolism. Where extrahepatic metabolism could be important, the incubation of compounds with blood or tissue homogenates may be appropriate.

At this stage, the various methods do not need to provide precise information on pharmacokinetic parameters or toxicological effects, but, ideally, should give relative values which can be used as a basis for lead identification or further structural searching.
**Lead Optimisation**

The aim of lead optimisation is to obtain good biological activity which is likely to translate to humans. Its success is increasingly likely to be based on the rational modelling of *in vivo* behaviour, as a result of the provision of information on metabolism, pharmacokinetics and toxicology much earlier in drug discovery than at present.

In early lead optimisation, the focus should be on characterising metabolism and pharmacokinetics. The number of compounds under consideration is now considerably reduced relative to lead identification, but the systems employed still need a capacity of tens of compounds per week. Intestinal absorption, tissue penetration, metabolism, stability and elimination are pharmacokinetic parameters which must be considered. Ideally, assessment of metabolism should include the determination of metabolic rates, since these can show great species variation. Human hepatocytes are especially relevant here. Other *in vitro* systems, such as the Caco-2 cell line which may be used to evaluate transcellular absorption, can also be helpful.

Knowledge of aqueous solubility, lipophilicity, pKa, protein binding and other physicochemical parameters is as essential in lead optimisation as it is in lead identification. The *in vivo* studies and PBPK modelling systems employed previously can also be applied to any new chemical derivatives.

Early indications of toxic potential and of target organs are very useful at this stage. Cytotoxicity data obtained with organ-specific cells can be employed and, as they become available, mechanistically based screening methods (for example, for immunotoxicity, nephrotoxicity, neurotoxicity and phototoxicity) will also be of value for lead optimisation purposes. The identification of toxic effects which are dependent on the particular chemical series or are pharmacologically related is of particular importance.

**Compound Selection**

Compound selection implies that drug discovery has been successful, and that a batch of compounds have emerged which are likely to be sufficiently safe and efficacious. Further selection will take place on the basis of other considerations, such as ease of synthesis and stability. Another important factor is the absence of significant toxicity in more than one species.

The use of experimentally determined *in vitro* metabolism kinetics in estimating the likely kinetic behaviour of a compound is an exciting new development (5, 6). Estimates of *in vivo* metabolic clearance can be integrated with data from PBPK models for different species to predict likely human pharmacokinetics. If this methodology is shown to be reliable, it will permit a more rapid selection of compounds for further development (7).

**The Integrated Use of Measurements and Models**

To achieve rapid drug development, active candidate compounds can now be appraised via an integrated programme of *in vitro* and *in vivo* studies, ideally initiated after a computer-based screening of their likely physicochemical and toxicological properties (8). The goal is not merely to identify which of a series of lead compounds has the most appropriate safety profile, but also to permit the early commissioning of Phase I clinical trials. The evaluation of a compound in healthy human volunteers will provide important data on tolerance, pharmacokinetics and possible pharmacodynamics, which can be rapidly fed back to the discovery team to help its search for lead compounds and/or aid in the design of the subsequent development programme. This approach is only feasible because of our increased understanding of biological mechanisms, and advances in computerisation and analytical instrumentation. It also means that compounds may now be initially de-selected without resorting to animal testing. For lead candidates, some animal testing will be necessary, at least for the foreseeable future.

**Physicochemical properties**

Consideration should be given to molecular weight (MW), physical properties (appearance, salts, etc.), lipophilicity (log P; log D — the log of the distribution coefficient at pH 7.4), pKa, solubility, chirality, and the likely formulation and route of administration. Quantitative structure-activity relationship
(QSAR) and computer modelling studies can be of value here, for example, in the assessment of absorption and/or barrier penetration.

**Computer-based systems/molecular modelling**

By using rule-based programs or neural networks, it is possible to predict, with increasing success, the potential toxic effects (relating to specific endpoints) associated with a particular chemical structure (for example, by using DEREK [Deductive Estimation of Risk from Existing Knowledge; 9], or the likely cytochrome P450 isoform that mediates metabolism.

Molecular modelling has developed slowly, since it is generally based on the crystal structure of bacterial cytochromes P450 (10), the only cytochromes crystallised to date. These computational programmes are being developed to provide either models of human enzyme substrate binding sites (11), or sites of metabolic instability based on frontier orbital electron densities (12). This approach has also been applied to predicting various forms of toxicity. The COMPACT (Computer Optimised Molecular Parametric Analysis of Chemical Toxicity) system employs modelling software to calculate the molecular and electronic structures of molecules, such as frontier orbital energies, which are of fundamental importance in describing the potential reactivities of molecules (13).

The current limitations of such systems should not be overlooked (for example, the cytochrome P450 models are based on bacterial enzymes). As yet, most computer models for predicting absorption, metabolism and toxicity have not been validated to any great extent (14).

**In vitro studies**

The in vitro studies conducted typically include metabolic and barrier penetration screens; these are primarily low-throughput systems. The metabolic screens used have mainly been hepatic microsomal, hepatocyte or slice incubations, primarily derived from rat and dog. Compounds can be ranked quickly in terms of their metabolic stabilities by using these methods, especially with respect to those that are rapidly turned over; the sites at which metabolism occurs can be identified with the use of qualitative mass spectrometry. This information can be fed back rapidly to the project team, so that efforts can be directed toward the introduction of functional groups which will alter the physical properties and/or make the molecules more metabolically stable.

Qualitative information on routes of metabolism can be obtained routinely from in vitro incubations. Furthermore, there is growing interest in the combined use of in vitro systems and biokinetic models to predict metabolic clearance in vivo (5). Conventional kinetic studies with in vitro systems can reveal the apparent maximum rate of metabolism (Vmax) and the substrate (drug) concentration required to give half the apparent maximum rate (Km). Under first-order conditions, Vmax and Km can be related to intrinsic clearance (Clint) by the relationship:

\[
Cl_{int} = \frac{V_{max}}{Km}
\]

By using this relationship, Michaelis-Menten parameters can be determined and the intrinsic clearance can be calculated. With the use of scaling factors, the hepatic clearance can be determined (15, 16), although with varying degrees of success depending upon the particular in vitro system used. To use in vitro data to give quantitative information, PBPK models can be developed for use in predicting the kinetics and toxicities of compounds (17).

Barrier penetration has been studied and assessed in vitro during the lead optimisation stage, by using either Caco-2 cells as a model of GI absorption (18, 19), or cerebral endothelial cells as a model of the blood–brain barrier (BBB; 20–23). These systems have not yet been fully validated for these purposes, and limited data exist for comparing in vitro and in vivo models of absorption (24) or brain penetration (20). However, cell-based systems have been used successfully to rank the ability of compounds to penetrate a membrane, and have provided a valuable insight into the behaviour of compounds. Furthermore, studies with artificial membranes for measuring penetration could lead to the development of a high-throughput system for use in the future.

A number of in vitro models have been used for the detection and characterisation of target organ toxicity (4, 25–27), of which
isolated hepatocyte cultures have been the most extensively studied. To date, hepatocyte cultures have not been used in primary screening, but have proved to be valuable for characterising mechanisms of toxicity at later stages of drug development. In addition, various non-cellular \textit{in vitro} methods have been proposed as screens for metabolically activated toxicants (28, 29) and for compounds that induce pro-oxidant states (30); these may provide starting points for the development of mechanistically based high-throughput screens for such compounds. Thus, it seems likely that the development of \textit{in vitro} screens for toxicity will proceed on two fronts: a) the use of cultures of relevant target cells; and b) the use of mechanistically based non-cellular systems.

Human material has not been used routinely in \textit{in vitro} discovery screening due to the amount of material which would be needed to satisfy all of the drug discovery projects. In addition, supply has been a problem in the past, as has confidence in the quality of the material used and, consequently, of the data produced. The issue of supply has begun to be resolved now that material is available commercially. The availability of a battery of human enzymes heterologously expressed in bacteria, yeast, insect cells and mammalian cells (31) provides the opportunity to produce large quantities of protein which can be used in high-throughput metabolic screens. However, given the significant requirement for throughput of compounds in discovery screening, cost is still an important consideration.

The \textit{in vitro} methods used during lead optimisation are intended to support internal decisions. It is therefore unnecessary for them to be subjected to formal, interlaboratory validation. It is, of course, necessary to have standardised procedures, and to validate the biological significance, as well as the reliability of the individual tests, for instance, by using known reference compounds. The main issue, however, is the relevance of the individual test and the specific test battery to the intended target of the compound which is to be optimised.

\textbf{In vivo studies}

Generally, the most promising compounds from \textit{in vitro} screens have been selected and their pharmacokinetic parameters evaluated in the rat or dog, in order to assess and optimise their kinetic profiles. Attempts should also be made to gain information about structure–toxicity or structure–pharmacokinetic relationships from these data. Although this is not possible in every case, such relationships (for example, those between physicochemical parameters such as partition coefficients, and metabolic clearance, or volume of distribution) may help in defining how chemical structures should be modified (3).

If the plasma clearance is high and is approaching liver blood flow, in any species, then the assumption is generally made that hepatic clearance is likely to be high. Excreta and plasma are examined for metabolites, and the structural information is passed back to the project chemists. If metabolic clearance is not a problem in a structural series, then limited oral studies are conducted in a single species, usually in the rat. These studies consist of giving a single dose, with limited sampling points, to a few animals. This reduces the numbers of animals required and increases efficiency and compound throughput.

Advances in mass spectrometry, particularly in the atmospheric pressure machine, have enabled a number of compounds to be co-administered to either the rat or dog simultaneously, and at very low doses. This approach has been used to good effect in producing pharmacokinetic data with particular emphasis on absorption. However, so far an insufficient number of compounds have been studied and the analytical limits of this approach have not yet been fully assessed. There has been an increase in the use of liquid chromatography–mass spectrometry for plasma analysis, rather than high performance liquid chromatography. The former has the advantages of automation, short analytical times, and multiple analysis after pooling samples.

When undertaking \textit{in vivo} studies, it is best practice to initially conduct small “pilot” investigations with only a few animals, and to base any decisions about the most appropriate way to proceed on the results obtained in these pilot studies. In general, the workshop participants supported the view that many current \textit{in vivo} pharmacokinetic studies used more animals than the minimum which would be required if physicochemical and \textit{in vitro} data were
used in a more structured manner. The use of such an approach is important, since it offers the possibility of reducing the numbers of animals used in pharmaceutical research and development without compromising safety.

**Specific Examples**

*Selection of macromolecular products for chemical development*

Soluble synthetic polymers can be covalently bound to therapeutic agents via linkers which are designed for site-specific cleavage within the body. The resultant macromolecular products are suitable for intravenous administration, and can be directed to specific organs, tissues or tumours. As the polymer-bound drug is inert, this considerably reduces any toxicity associated with the drug (32).

A polymer conjugate was developed, comprising the water-soluble copolymer, N-(2-hydroxylpropyl)methacrylamide (HPMA), linked to doxorubicin via the tetrapeptide spacer, gly-phe-leu-gly. This compound is called PK1 and has a molecular weight of approximately 30,000 Daltons and a doxorubicin content of approximately 10% (by weight). The conjugate is only taken into cells by endocytosis, providing the opportunity for lysosomotropic drug delivery and thus bypassing the mechanisms of resistance associated with the plasma membrane efflux pump, P-glycoprotein. Doxorubicin must be liberated from the conjugate by lysosomal thiol-dependent proteases before it is able to exert its cytotoxic effect, and it is believed that this happens intracellularly. A complex mechanism of action is involved, which results from pharmacokinetically guided design (33).

The results of the Phase I clinical evaluation of PK1 (FCE 28068) have recently been reported, and show that the behaviour of the conjugate in humans (reduced toxicity, suitable pharmacokinetics) was similar to that observed preclinically, and that polymer-doxorubicin displays anti-tumour activity in man (34). This supports the approach taken for the rational design of polymeric anti-tumour agents (32, 33).

Polymeric anti-cancer agents are complex macromolecular drugs comprising the polymer backbone, the polymer drug-linker, and a bioactive anti-tumour agent. Therefore, they can only be captured by cells by endocytosis, so an appreciation of their pharmacokinetics at the cellular and the whole-organism level is essential. To achieve an improved therapeutic index in vivo, it has been shown that it is essential to study the following factors systematically and to feed the parameters back into the molecular design (Figure 1):

1) biocompatibility (or toxicology) of the proposed carrier — this can largely be undertaken in vitro and is suitable for automation by using high-throughput screens;

2) the stability of the polymer-drug linker in the environments it will encounter, extracellularly and intracellularly (plasma, lysosomal) — this is suitable for automation by using model enzyme systems in vitro;

3) rates of endocytosis — in vitro analysis is possible; and

4) biodistribution and pharmacology in vivo.

Experience gained during the transfer of this novel approach from the laboratory to the clinic allowed certain general comments to

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**Figure 1: Rational design of polymeric anti-cancer agents**

**Synthetic Chemistry**

- Monomers
- Polymer linkers
- Characterisation

**Biological Evaluation**

- Biocompatibility
- *In vitro* rate of drug release
- Cellular/whole body pharmacokinetics
- Pharmacology
be made with regard to the importance of early pharmacokinetic evaluation in the selection and optimisation of lead candidates for in vivo and/or clinical study. In addition, early pharmacokinetic data are essential, to allow effective in vitro/in vivo human correlations to be made.

Although this research has focused on the design and development of polymer therapeutics, the main issues are directly relevant to any macromolecular drug. The last decade has seen a steady stream of protein and peptide drugs coming to the market, and there are currently great hopes for compounds which might be called “gene therapeutics”.

*Caco-2 monolayers in experimental and theoretical predictions of drug transport*

During the last few years, the use of intestinal epithelial cell lines such as Caco-2 and HT29-H has increased dramatically in many research fields, including the pharmaceutical sciences. The cell lines are now routinely cultured as monolayers on permeable filters for studying the transepithelial transport of drugs (18, 19, 35, 36).

Comparison of drug transport in Caco-2 monolayers with intestinal drug transport in vivo indicates that the monolayers can be used to predict drug transport by different pathways across the intestinal epithelium (24, 37–41). The best correlation to the in vivo situation is obtained for drugs transported by the passive transcellular route (42). The passive paracellular route is less permeable in the cell monolayers than in vivo, but the data so far indicate that the selectivity of this pathway is comparable to the in vivo situation (43). From these results, it can be concluded that Caco-2 monolayers can be used to identify drugs with potential absorption problems, and possibly to select drugs with optimal passive absorption characteristics from series of pharmacologically active molecules generated in drug discovery programmes. The absorption of drugs transported via carrier-mediated mechanisms can probably also be predicted in some, but not in all, cases. However, to confirm this, a more extensive characterisation of each active transport mechanism needs to be performed. Moreover, it is evident that there is a need for standardisation of Caco-2 cultures. Direct comparison of drug permeabilities obtained in different laboratories will only be possible if the same Caco-2 cell population, cell culture and experimental conditions are used (44, 45).

Many attempts have been made to explain and predict passive drug absorption directly from the properties of a particular drug molecule. In these studies, single physicochemical properties, such as log P (46), hydrogen bonding capacity (47) or desolvation energy (48), have been correlated with the intestinal absorption rate or the cell membrane permeability. One advantage of using single parameters for this purpose is that they are relatively easy to determine experimentally, or to derive from theoretical models. However, the relative importance of these properties will vary from one chemical type to another, so only approximate correlations can be obtained with single physicochemical properties.

Initial studies with a new theoretical method based on molecular surface properties suggest that the dynamic polar surface area, calculated by using molecular modelling, could be an interesting alternative for the prediction of drug absorption (49). The dynamic polar surface area correlates well with drug permeability in Caco-2 monolayers and excised intestinal segments for the limited series of compounds investigated to date. This suggests that Caco-2 monolayers could be used as a convenient reference model for theoretical predictions of drug absorption.

Very powerful methods have recently been developed for the combinatorial synthesis of large libraries of peptides and organic compounds. Powerful new methods have also been developed for the high-throughput screening of pharmacological activity. As a result, large numbers of compounds with promising pharmacological activities are being obtained. This has increased the demand for screening methods for oral drug absorption (50), which suggests that the interest in cell culture models for experimental and theoretical predictions of drug absorption will continue to increase.

*The in vitro blood–brain barrier model for investigating drug transport to the brain*

The isolation and culture of brain microvascular endothelial cells (BMEC) has had a major impact on research on the transport of drugs across the BBB into the brain, particularly at the cellular and sub-cellular levels (21, 51–53). BMEC can be isolated from human,
bovine, porcine and rat brains by using a completely non-enzymatic procedure (22), a combined mechanical and enzymatic method (23), or a solely enzymatic procedure (21). The non-enzymatic procedure may reduce the loss of surface molecules which can occur during treatment with enzymes, particularly with non-specific proteases. Cells are grown to confluency on cell culture filters in the presence of astrocyte-conditioned medium or astrocytes. The use of astrocyte-conditioned medium (22, 23) or co-culture with astrocytes (22) improves the maintenance in vitro of properties associated with the BBB in vivo (54, 55).

It is important that the in vitro systems to be used are well-characterised morphologically, biochemically and functionally. Cultured BMEC monolayers are useful for studying a wide range of important processes, for example: a) drug transport across the BBB, including passive hydrophilic (paracellular) transport, and passive lipophilic and carrier-mediated/receptor-mediated (transcellular) transport; b) drug metabolism; c) the effects of specific compounds and conditions on the functionality of the BBB, both in normal situations and in disease states; d) visualisation of drug transport routes through confluent BMEC monolayers by using confocal laser scanning microscopy; and e) measurement of the trans-endothelial electrical resistance. It is important to investigate whether disease states change the properties of the BBB and, as a result, affect the transport of drugs to the brain. Inflammatory conditions, which may occur, for example, in multiple sclerosis, Alzheimer’s disease, AIDS-related dementia, meningitis and encephalitis, can be induced in vitro by the addition of lipopolysaccharide. Under such disease conditions, BBB permeability, functionality and drug transport can be studied (56, 57).

Conclusions and Recommendations

Physicochemical properties and structure-activity relationships

Significant problems remain in the calculation of log P, pKa and log D, particularly when the very large numbers of compounds likely to be of interest in the early screening stage as a consequence of high-throughput chemistry are taken into account. Comparative studies on the algorithms available for log P, pKa and log D estimation are required. Validation of these methods is also needed. The current methods for estimating solubility are inadequate and labour-intensive, and they have not been standardised.

If log P, pKa and log D and other QSAR parameters are known, some important distribution parameters, such as GI absorption, protein binding and BBB permeability, can be estimated adequately for use at the early compound screening stage. However, further validation of this approach with a wider range of chemical structures is required.

Predictive models for toxicity and metabolism are less well developed. Further methodological developments for the validation of knowledge bases for expert system approaches are necessary (14), while with numerical calculations, such as modern pattern recognition methods, comparative studies for establishing their predictive performance are essential.

It will also be necessary to develop models which integrate and evaluate information about the component pharmacokinetic processes. Such overview models could be developed from data which are available on existing compounds but, at present, information is only readily available for a relatively small number of compounds. The question of which physical properties determine the distribution of macromolecules, and how they might be estimated, is still poorly understood, and there is a need for basic modelling to address this question.

The advantages and disadvantages of the methods currently used for determining physicochemical properties are outlined in Table I. The following recommendations are made:

1. Expert systems or other models which can generate toxic or metabolic alerts based on historical precedent would be very valuable in supporting early decisions. The further development and validation of these methods should be supported.

2. Physical properties, such as log P, pKa and log D, provide essential information, and comparative studies of the predictive methods available should be carried out to identify the best methods for supporting early pharmacokinetic studies.

3. Physical property modelling of macromolecular distribution should be undertaken.
Table I: Methods used for determining physicochemical properties

<table>
<thead>
<tr>
<th>Methods</th>
<th>Use</th>
<th>Advantages</th>
<th>Disadvantages</th>
<th>Future needs</th>
</tr>
</thead>
<tbody>
<tr>
<td>clogP, prolog P</td>
<td>log P estimation</td>
<td>fast; computer-based</td>
<td>unreliable for some structures</td>
<td>mature area; little development needed</td>
</tr>
<tr>
<td>PKALC</td>
<td>pKa estimation</td>
<td>fast; computer-based</td>
<td>unreliable for some structures</td>
<td>further development possible</td>
</tr>
<tr>
<td>Solubility and physical property measurements</td>
<td>well-established, reliable experimental methods available</td>
<td>costly and time-consuming</td>
<td>automation, miniaturisation and improved analytical methods</td>
<td></td>
</tr>
</tbody>
</table>

Table II: Methods used for assessing toxicity

<table>
<thead>
<tr>
<th>Methods</th>
<th>Use</th>
<th>Advantages</th>
<th>Disadvantages</th>
<th>Future needs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Databases</td>
<td>prediction alert</td>
<td>uses prior knowledge</td>
<td>limited accessibility to historical data</td>
<td>improve accessibility</td>
</tr>
<tr>
<td>Expert systems</td>
<td>prediction alert</td>
<td>analyses SAR from a number of perspectives</td>
<td>accuracy; limited expertise; limited endpoints</td>
<td>improve and refine</td>
</tr>
<tr>
<td>Cellular toxicity models</td>
<td>cell-based toxicity; target cell identification</td>
<td>amenable to greater efficiency; fairly rapid; does not require much compound</td>
<td>need to prove relevance</td>
<td>maintenance of organo-typic function; definition of a panel of meaningful cells</td>
</tr>
<tr>
<td>Mechanistic models</td>
<td>screens for common toxic effects</td>
<td>high-throughput; gives scope for chemical modification; species differences</td>
<td>knowing relevance of individual mechanisms</td>
<td>more basic research</td>
</tr>
<tr>
<td>In vivo</td>
<td>identification of targets; definitive assessment</td>
<td>integrated whole-body response; relevance accepted</td>
<td>species extrapolation; time-consuming</td>
<td>better markers; transgenic animals; integration of data from non-animal methods in design</td>
</tr>
</tbody>
</table>
Assessing toxicity

The key issues in assessing toxicity are: a) target organ identification; b) the identification of general (that is, not organ-specific) targets; c) species differences; d) better understanding of mechanisms of toxicity; e) improving the availability of human material for in vitro studies; and f) improving the general accessibility of databases.

The advantages and disadvantages of the methods currently used for assessing toxicity are outlined in Table II. The future requirements for toxicological assessments at an early stage in drug discovery are given in Table III. It is recommended that:

1. Support should be increased for the development of toxicology databases. This would be greatly facilitated if industry could be persuaded to release more information for use by others.

2. There should be greater emphasis on the identification of common and relevant target cells and the establishment of suitable cell-based toxicity screens. Relevant target organs should be identified in collaboration with industry.

3. Basic research into general mechanisms of toxicity, markers for specific toxic effects (for example, stress responses, free-radical damage, covalent binding), and their relevance for predicting toxicity, should be encouraged. Mechanistically relevant cellular systems and markers should then be integrated into cell-based toxicity screens.

Assessing metabolism

A key issue is whether metabolism screening needs to be conducted with biological systems, or whether it can be accomplished by

<p>| Table III: Future requirements for undertaking toxicological assessments at an early stage of drug discovery |
|-----------------|-----------------|-----------------|-----------------|</p>
<table>
<thead>
<tr>
<th>Phase</th>
<th>Needs</th>
<th>Uses</th>
<th>Future trends</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lead structure identification</td>
<td>— high-throughput screening</td>
<td>increase probability for benign toxicological profile; exclude toxic structures</td>
<td>new predictive markers for toxicity — stress genes — cell cycle markers expert systems</td>
</tr>
<tr>
<td>— secondary screens</td>
<td>limited cytotoxicity testing</td>
<td>mechanistically-based screens</td>
<td></td>
</tr>
<tr>
<td>Lead optimisation</td>
<td>identify critical targets; reduce toxicity on these targets</td>
<td>exploratory toxicity for target class-related/structure-related toxicity; in vitro screening on selected targets</td>
<td>better in vitro screens — recombinant cell lines — mechanistic markers — new techniques (PCR, mRNA mapping)</td>
</tr>
<tr>
<td>Candidate selection</td>
<td>predict all relevant targets; assess human risk; select the best candidate</td>
<td>comprehensive in vitro and in vivo test battery; mechanistic studies; definitive in vivo studies</td>
<td>transgenic animals; better ex vivo markers</td>
</tr>
<tr>
<td>System</td>
<td>Status</td>
<td>Use</td>
<td>Advantages</td>
</tr>
<tr>
<td>-----------------------</td>
<td>-----------------</td>
<td>--------------------</td>
<td>-------------------------------------------</td>
</tr>
<tr>
<td>Recombinant systems</td>
<td>validated</td>
<td>lead optimisation</td>
<td>pure system</td>
</tr>
<tr>
<td>Microsomes</td>
<td>established</td>
<td>lead optimisation</td>
<td>proven utility; ready storage; well-characterised</td>
</tr>
<tr>
<td>Isolated cells</td>
<td>established</td>
<td>lead optimisation</td>
<td>integrated cellular system</td>
</tr>
<tr>
<td>Slices</td>
<td>evolving</td>
<td>lead optimisation</td>
<td>ease of preparation; intact architecture</td>
</tr>
</tbody>
</table>
### Table V: Biological systems used for assessing permeability

<table>
<thead>
<tr>
<th>Systems</th>
<th>Use</th>
<th>Advantages</th>
<th>Disadvantages</th>
<th>Future needs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Membrane vesicles</td>
<td>membrane transport; qualitative interactions</td>
<td>simple</td>
<td>poor correlation</td>
<td>metabolism</td>
</tr>
<tr>
<td>Isolated cell systems</td>
<td>quantification of intracellular distribution; active transport systems</td>
<td>rational design for (macromolecular) drugs</td>
<td>time-consuming</td>
<td>standardisation; improved analytical methods</td>
</tr>
<tr>
<td>Cell monolayers, tissue sections and loops</td>
<td>penetration; transport</td>
<td>controlled system; more physiological</td>
<td>variability; time-consuming</td>
<td>validation</td>
</tr>
<tr>
<td>Blood–brain barrier with or without astrocytes</td>
<td>transport; metabolism</td>
<td>routine cellular system</td>
<td>simplified system; not quantitative; extrapolation</td>
<td>include pericytes; standard reference compounds</td>
</tr>
</tbody>
</table>
using expert systems which would identify and rank possibilities. The current situation requires metabolic properties to be optimised by using a biological system, and a decision is taken about whether this should incorporate human tissue or tissues from the species to be used in any subsequent in vivo studies. Both metabolic route and metabolic rate should be determined.

It is likely that some combination of biological and expert systems would be the most productive approach, in combination with the use of PBPK models. One problem is that the design of studies and interpretation of the data they provide are currently dependent upon relatively few individual experts and the confidential databases to which they have access.

The advantages and disadvantages of the systems currently used for investigating metabolism are outlined in Table IV. It is recommended that:

1. Research aimed at the provision of more recombinant systems, with a greater range of diversity, should be encouraged. These systems must be fully characterised.
2. Attention should be focused on the circumstances in which the use of microsomal and/or whole cell systems is appropriate.
3. An objective evaluation should be conducted on the comparative utility of hepatocytes and liver slice preparations.
4. Procedures should be established at the European level for the safe, regular and ethical supply of human livers of high quality.
5. More effort should be invested in the development and optimisation of various types of techniques which could improve the preservation of metabolic functions in in vitro preparations, including cryopreservation and the use of culture matrices and various culture media.
6. Effort should be invested in the further development of PBPK modelling as a means of improving the usefulness of data provided by in vitro test systems.

Assessing permeability

A numbers of key factors must be considered when permeability is to be assessed, including the proposed route of administration of the drug, the target site, primary and secondary barriers, and potential sites of toxicity. The nature of the compounds under investigation is also very important; for example, whether they are low molecular weight or macromolecular (peptides, proteins, oligonucleotides or polymers) should be taken into account.

The advantages and disadvantages of the model systems currently used for assessing permeability are outlined in Table V. It is recommended that:

1. Standard procedures should be developed and validated for the model systems used to monitor penetration/metabolism at barrier sites and at the target cell level.
2. In vitro models should be used for studying GI and BBB permeabilities, especially for compounds with values of log P and log D at the extremes of their distributions.
3. Coser collaboration, including the sharing of databases at the European level, would lead to improvements in the biological systems and other models needed.
4. A workshop should be organised on the use of Caco-2 cells and other artificial and cell-based systems for assessing permeability.
5. A workshop should be held on the early use of cell models and in vivo pharmacokinetic models for the rational design and selection of macromolecular drugs.

References


