Early ADME/Tox studies and *in silico* screening

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The first day of the IBC Life Sciences *Early ADME/Tox Studies and In Silico Screening* symposium (4–5 October 2001, The Ambassador Hotel, London, UK) provided a comprehensive coverage of the application of *in vitro*, genomic and data management systems in the evaluation of drug properties.

The blood–brain barrier

The first session began with Joan Abbott (Department of Physiology, King's College London, UK), who reviewed *in vivo*, *in silico* and *in vitro* approaches to the evaluation of drug permeability across the blood–brain barrier (BBB). Key factors for the extent of drug penetration into the CNS were identified as BBB permeability, protein binding, aqueous solubility, intraspinal fluid distribution, bulk flow (fluid movement) and sink action (distribution of drugs) of cerebral spinal fluid and elimination from the CNS. For *in vivo* studies, CNS penetration measured using single-dose injection and at a single time-point was inaccurate. Measuring brain and plasma drug concentrations using multiple time-points after single-dose injection and determination of penetration using plasma and brain 'area under curve' values is more accurate. *In vitro* BBB models include animal and human brain endothelial cell lines, as well as human colon carcinoma cells (Caco-2). The porcine brain endothelial cell model was the model of choice because it shows good correlation with *in vivo* BBB permeability. Data correlating the *in vivo* pharmacological activities of barbiturate homologues and lipophilicity were shown; high lipophilicity did not always correlate with CNS penetration. Lipophilicity is important for partitioning of the drug into the endothelial cell membrane, and hydrogen-bonding potential is important for dissociation of the drug from the aqueous phase of the plasma.

ADME/Tox and human-based systems

Jacques Migeon (Cerep, Paris, France) spoke on high-throughput ADME/Tox and introduced the Cerep BioPrint™ model, which correlates chemical structure with ADME/Tox drug properties through molecular descriptors and empirical results from *in vitro* and *in vivo* experimentation. Using results from 1000 compounds, a good correlation was demonstrated between permeability across TC7 (a clone of Caco-2) cells and the percentage of oral absorption. Metabolic stability data using human liver microsomes and the S9 microsomal fraction showed that compounds with low metabolic stability generally had low bioavailability. Migeon believes that BioPrint data should eventually help build an *in silico* model for the prediction of drug properties.

The use of human-based experimental systems, such as human hepatocytes, in the early evaluation of human drug properties was discussed by Albert Li (In Vitro Technologies), who believes that results with laboratory animals alone are inadequate. The use of human *in vitro* systems and *in vivo* animal models could be a powerful combination in the selection of clinically successful drug candidates. Experimental data on the differences between the rat and man in drug metabolism (e.g. 7-hydroxylation is only observed in man), and enzyme induction (e.g. rifampin is an inducer of CYP3A only in man), emphasized the importance of understanding the limitations of *in vitro* systems.

Intact human hepatocytes allow drug distribution between extracellular and intracellular compartments and can be used to model intracellular effects (e.g. P450 inhibition) based on extracellular drug concentration (e.g. plasma). The presence of complete drug metabolizing enzymes and cofactors means that metabolic stability studies should be performed with intact hepatocytes. For drugs that are metabolized by direct conjugation the use of liver microsomes provides an erroneous estimate of metabolic stability and metabolite profiles. The application of cryopreserved human hepatocytes from multiple donors for drug toxicity evaluation showed that hepatotoxic drugs were cytotoxic to human hepatocytes using a variety of endpoints for viability.

Automated sample preparation and cell-based assays

Desmond O'Connor (Merck Sharp and Dohme, The Neuroscience Research Centre, Harlow, UK) described the Discovery PK program in the Merck Neuroscience Research Centre, which serves to select compounds based on pharmacokinetics (PK) for further development, and the Biomek 2000 automated liquid-handling station for automatic sample preparation. To increase the throughput of MS analysis, High Throughput MS–MUX, which employs a rotating selector to enable analysis of samples from multiple HPLC systems, was compared with staggered injections,

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which enables sampling from multiple HPLC at different times: staggered injection was reported to be the superior method. The automation of MS–MS method development using commercial software could optimize important MS parameters, such as parent- and daughter-ion molecular weight, cone voltage and collision energy, requiring only MW and 1–10 ng μl^{-1} solution as input.

High throughput cell-based assays, for early toxicity assessment, were discussed by Wei-Wei Li of Berlex Biosciences (Richmond, CA, USA). Early toxicology is the reduction of toxicity through the application of cell-based toxicity assays, gene expression assays, *ex vivo* enzyme markers and mutagenicity assessment. A cell-based cytotoxicity assay using Cytostar-T™ technology was described, which quantifies ^{14}C-thymidine uptake into cells cultured on Cytostar-T scintillation plates; the radioactivity being read directly using a scintillation counter. Results with bioluminescence, colorimetry and fluorescence were also shown. Data management, using the *in vitro* Tox Database was discussed, where each drug is classified by name, therapeutic indications and cytotoxicity results.

Reporter and biomarker genes

Peter Bromley (GeneTox and Gene Control SA, Geneva, Switzerland) described the use of *in vitro* and *in vivo* reporter gene assays in early predictive toxicology. The GeneTox™ 'Stress Reporter Gene Assay' involves the hsp70 family of genes as reporter constructs, fused with genes encoding enzymes that can be measured easily, such as luciferase or GFP. The StressTox™ Assay is a proprietary system of GeneTox based on a specially engineered hsp70 sensor, whose expression is inducable. The sensor–reporter constructs are expressed in transgenic animals, which are also used as *in vivo* toxicity tests. Primary cells from the kidney, liver, lung, skin and brain, as well as stem cells, are isolated from these animals and used for *in vitro* toxicity assays. Using HeLa cells expressing the Hsp70 promoter–catalase reporter gene construct, good correlation was observed between *in vitro* and *in vivo* acute toxicity.

Finally, the identification of predictive biomarker genes for kidney toxicity was described by Philippe Alen of Phase-1 BioResearch (Zwijnaarde, Ghent, Belgium). The Phase-1 Rat Toxbank® contains a molecular toxicology database from 32 compounds with data including gene expression, histopathology images, blood serum chemistry, urine analysis and hematology obtained from laboratory rats. The expression of >700 toxicity relevant genes was evaluated and, for each compound, there were >36 gene expression profiles, containing >25,000 gene-expression data-points. Data of kidney toxicity using known nephrotoxicants (e.g. cisplatin, hydroxyurea, lipopolysaccharide, puromycine and tetracycline) was obtained, and kidney histopathology scores were correlated with gene expression. The overall levels of gene expression correlated well with histopathological changes. Based on this correlation, indicator genes for kidney damage could be identified and used as a tool to identify kidney toxicants.

Modelling the human body

The second day of the conference focussed on *in silico* approaches to the prediction of ADME/Tox properties and PK of compounds in man. The need for efficient prediction of these properties early in the drug discovery process was emphasized. Indeed, the high attrition rate of compounds in development is largely a result of inadequate ADME/Tox properties [1]. The presentations described methods for overcoming these difficulties, based on computational approaches.

Three approaches to modelling the human body, to predict its interaction with a compound, were outlined by Willliam Bains (Amedis Pharmaceuticals, Royston, UK). The *in silico* approaches discussed thereafter can be understood within the context of these three categories.

The body as a machine – high level mechanistic models. This approach treats the body as a set of integrated biological processes that can be modelled mathematically. The assumption is that the key parameters of the processes are known and these models require the basic properties of a molecule (e.g. solubility, metabolic fate or cell permeability) to be provided as inputs.

The body as a polymer – molecular mechanistic models. The body can be viewed as a collection of molecules interacting on the atomic scale. Hence, individual mechanisms can be determined using molecular modelling techniques but this approach can only be applied to a well-characterized molecular process.

Man as enigma – data mining approaches. The processes underlying a PK or ADME/Tox property of a molecule can be treated as a 'black box'. The relationships between the structure of a molecule and the output of the black box can be probed by statistical methods. This requires data on a large set of molecules, which can be used to 'train' a mathematical model to fit the observations. The concept of 'chemical space' is important in this approach as a model can only provide reliable predictions for molecules lying in chemical space represented in the dataset used to train the model, so the greater the diversity of compounds used to train a model, the better.

Disease modelling

Michael Liebman (University of Pennsylvania Cancer Center, PA, USA) discussed the modelling of disease not as a state, but as a sequence of responses of the body that might be observed as symptoms, providing an excellent example of the first of the approaches outlined by Bains. By representing a disease as a set of linked processes, which can be modelled using dynamic or stochastic techniques, early indicators of a disease, and possible targets for pharmacological intervention, can be identified. Liebman

gave examples of the application of this approach to the coagulation process [2] and ageing and disease in the endocrine system.

Oral bioavailability

David Leahy (Cyprotex, Manchester, UK) presented the application of similar numerical simulation methods to the prediction of PK properties. A multi-compartment model of the body can be used to construct a set of equations that describe the distribution of a compound, leading to simulation of the timecourse of the plasma concentration of a drug. The required model inputs include predicted tissue partition coefficients and intrinsic clearance obtained from human liver microsomes or hepatocytes. A numerical model for oral bioavailability simulates the timecourse of the dose distribution between different intestinal compartments and plasma. Model parameters for a particular compound, including the effective permeability and the volume of distribution, can be predicted from the structure of the compound. However, the contribution to bioavailability because of first-pass metabolism needs to be estimated from *in vivo* clearance data, which will limit the applicability of this model in the early stages of drug discovery. The advantage of these models is that detailed questions can be posed and simulated, such as the effect of different dosing regimes or the presence of interacting drugs.

A data-driven approach to the prediction of oral bioavailability was discussed by William Bains, who described an 'evolutionary' approach to selecting a good set of parameters for a mathematical model, linking a set of molecular descriptors to oral bioavailability. Two genetic algorithms were presented, based on artificial neural networks and genetic programming. These techniques enable the construction of non-linear models, which are often necessary to capture the complex relationships between the variables and target values. The bioavailability models obtained using these two methods were very similar, although there was not a good correlation between predicted and observed bioavailability. Notably, neither of the models predicted any compounds to have bioavailabilities below ~15%. This is probably a result of a bias in the data available to train the model towards high bioavailability compounds and is a general problem in developing such models, as large datasets with a uniform distribution in target value are often not available. Classification models based on these methods appeared to perform better, similarly to the published Topliss model [3].

Another model for the prediction of human oral bioavailability was presented by Daniel Norris (Lion Bioscience, San Diego, CA, USA), which was based on a combination of the approaches described by Bains. Modules describing the absorption and first-pass metabolism processes were trained to fit the observed *in vivo* data for a set of compounds and combined into a single physiological model. The inputs to these modules, for each compound, consist of an extensive set of *in vitro* data, including bidirectional Caco-2 permeability, solubility, hepatocyte and protein binding data. The resulting model showed a significant scatter, with a mean precision of 14.1% for the predicted fraction reaching the hepatic vein. However, this is less than the mean reported precision of 25.5% in corresponding *in vivo* data.

Drug metabolism

Matthew Segall (Camitro, Cambridge, UK) described the remaining molecular approach to predicting ADME/Tox properties, which relies on a mechanistic understanding of a process at a molecular level and requires detailed experimental results to build a model. However, the resulting models are transferable and require no inputs other than the structure of the compound. The transferability and accuracy of this approach is best if the model is based on quantum mechanics. Segall described the application of first principles quantum mechanical modelling to metabolism by cytochrome P450 [4], with the aim of gaining a better understanding of the mechanism of metabolism. The resulting models accurately predict the regioselectivity of metabolism by human cytochrome P450s 3A4, 2D6 and 2D9.

Conclusion

Human oral bioavailablility is a difficult property to model because multiple processes in the body contribute to the bioavailability of a compound and there is little high quality data on a broad range of chemical diversity. Greater success has been obtained for models of other ADME/Tox properties, such as drug metabolism, human intestinal absorption, BBB penetration and solubility. It is clear that a combination of the approaches discussed at this conference will ultimately be required to fully characterize the PK of a compound from molecular structure alone. Individual ADME/Tox properties could be predicted from molecular or data-driven models, based on molecular structure alone. These can serve as inputs to simulations that could predict PK parameters, such as bioavailability, clearance and volume of distribution. This will help to realise the goal of fully characterizing the PK of a compound early in the drug discovery process.

References

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