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Other cannabinoids in development for MS include Pharmos' early-stage CB_2 receptor agonist, HU308, and Pharmaxis' PXS2000, which is intended to retain the beneficial properties of cannabis without its psychotropic effects.

Cannabis has a profound effect on appetite stimulation – 'the munchies' as it is termed by recreational users. Studies as early as 1933 performed by a military committee illustrated increased food intake in subjects taking marijuana. In the USA, cannabis has been used in patients with AIDS to ameliorate the cachexic symptoms of the disease. Solvay and Nektar are currently collaborating to develop an inhaled formulation of dronabinol (currently undergoing Phase I development) for the treatment of wasting and weight loss in HIV patients.

The future of cannabinoids

The benefits of cannabinoids are extensive, with compounds under development for numerous indications other than those mentioned, including cough, bulimia, inflammation, Parkinson's disease, Tourette's syndrome and schizophrenia (Figure 4). It is therefore unsurprising that entire pharmaceutical companies, such as GW Pharmaceuticals, are focusing their R&D efforts on developing therapeutic cannabinoids. Enthusiasm about the therapeutic use of cannabinoids is curtailed only by consideration of the side effects of the drugs, which include psychomotor and cognitive impairment, postural hypotension, anxiety and panic attacks, acute psychosis and paranoia, palpitations and tachycardia. With the majority of cannabinoids in preclinical stages, it seems that the future of cannabis as a therapeutic agent remains uncertain, even amid its increasing popularity as a recreational drug.

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In vitro assays: crystal balls or random guesses?

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In Vitro *Screens in Drug Metabolism*
Orlando, FL, USA
13–14 December 2004

Organizers:
Cambridge Healthtech Institute

One of the central challenges in drug metabolism and pharmacokinetics is that of prediction, extrapolating data generated from *in vitro* models to the human response as early as possible. This is a problem that has been addressed by many review articles and conference sessions. The Cambridge Healthtech Institute presented its first meeting tackling this subject, In Vitro *Screens in Drug Metabolism*, on 13–14 December 2004 in Orlando, FL, USA. Would this conference produce any additional information to what is an information rich, but solution poor, subject? The combination of an interesting programme, the hot Florida sunshine and a hotel in the middle of Disney World ensured that the meeting was a success, both scientifically and socially!

Assay development with novel approaches to drug metabolism

Underscoring any predictive *in vitro* system must be the development of novel assays that correctly model the *in vivo* system and, preferably, increase knowledge of *in vivo* biology. The first session of the conference attempted to bring together emerging and validated technologies for *in vitro* screens, ranging from cytochrome P450 (CYP) turnover to novel cell lines. The regulation of CYP turnover was described by Amit Banerjee (Wayne State University, MI, USA). CYP expression is central to the metabolic processing of many chemicals, and thus CYP turnover could be an important predictor of metabolic capacity. Banerjee presented data showing the identification of specific components of the ubiquitination cycle that regulate CYP turnover, and hence ultimately control the level of CYP-mediated metabolism. *In silico* modelling was used to produce small molecule inhibitors of this process, resulting in a new set of chemicals that represent a promising novel method for regulating CYP-mediated metabolism.

Continuing the theme of small molecule inhibitors, Pierre-Yves Abecassis (Sanofi Aventis) described HTS for mechanism-based enzyme

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inhibitors (MBEI). In contrast to the research presented by Banerjee, Abecassis was working towards screening out inhibitors rather than developing them; MBEI of CYPs represents a major cause of drug–drug interactions in the clinic. To predict fully the consequences of MBEI, it is important to answer several questions – which CYPs are inhibited? How potent is that inhibition? Is the inhibition reversible? Abecassis presented data on a statistical model developed at Sanofi that enables researchers to answer these questions. Through the use of a simple 96-well assay, researchers at Sanofi are now able to assess the IC_{50} for any particular compound over a range of time points. Because MBEI shows an increase in potency of inhibition with incubation time, it is also possible to separate MBEI and non-MBEI events.

The foundation of many *in vitro* screens is the cells that the screen is performed in, every scientist appears to have their preference, be it cell line or primary tissue, and their own way of treating these cells such that they respond in an '*in vivo*-like' manner. This has proved a particularly thorny issue to resolve. Dirk Roymans (Johnson & Johnson) and Andrew Parkinson (Xenotech) championed two of the opposing camps, presenting data on the use of cryopreserved primary hepatocytes and immortalized cell lines, respectively. Although primary human hepatocytes are often seen as the 'gold standard', batch-to-batch variability and a lack of simple cryopreservation is a problem. Recent advances have increased the consistency and utility of cryopreserved human cells, and Roymans presented various markers for the cryopreserved human hepatocytes used within his group. Roymans showed that these cells exhibit many of the features associated with fresh human hepatocytes and could thus now represent a real alternative. Although Parkinson agreed with many of the comments made by Roymans, he outlined another factor. Parkinson showed that there appears to be a 'ceiling' for enzyme expression, the visible effect of which is the often-seen batch-to-batch variation in induction. Because the ceiling for expression is fixed, then the increase is set by basal expression levels; as basal expression varies between samples, so does the increase observed. Parkinson pointed out that whereas higher basal expression levels are desired to optimize the modelling of the *in vivo* situation, a screening assay with lower basal expression might be preferable because such an assay would increase the dynamic range available. Parkinson went on to describe a novel cell line marketed by Xenotech, Fa2N-4, that has low basal expression levels, and also appears to respond to a number of different inducers of CYPs. Wider testing will show the utility of this cell line, but it does seem a promising tool for the *in vitro* biologist.

Finally, Mike Mohutsky (Eli Lilly) outlined a method to increase the data generated from each batch of primary human hepatocytes using cassette-doing, thus increasing the utility of the cells and the cost-effectiveness of the experiments. Using this method, the expression levels of up to three CYPs can be measured simultaneously in a single preparation of hepatocytes. The data presented showed such an approach has merit, increasing the data produced from a precious resource.

Understanding the role of metabolism in drug discovery and development

Metabolism data for a drug, and what should be done with this information, were also discussed. The presentations highlighted the various strategies used by different companies to interpret metabolism data, and which bits of data researchers thought of as important. Mario Monshouwer (Pfizer) gave a general overview of the area, showing how adverse drug reactions caused by reactive metabolite formation result in a significant number of drug withdrawals. A combination of nuclear receptor reporter gene assays, MBEI screens and CYP-expressing cell lines are being used to gain information on potential problems at the earliest stage possible in discovery and development.

Drugs only work if they get to the target organ, and Richard Morrison (Schering Plough) gave an interesting presentation on his approach to predicting the pharmacokinetics of a compound in humans as early as possible. A whole raft of techniques from hepatocyte clearance to nuclear receptor activation assays were discussed, along with how this information can be extrapolated to *in vivo*. Unfortunately, as with all complicated systems, the conclusion is that extrapolation is as much an art as it is a science, with both success and failure observed in accurate extrapolation.

Also under discussion was the identification of metabolites, be they of pharmacological or toxicological relevance. Reza Anari (Merck & Co), Zhengyin Yan (Johnson & Johnson) and Elisabeth Graham (Memory Pharmaceuticals) gave their experiences in this challenging field. Anari discussed *in silico* identification of metabolites using the MDL browser (Elsevier MDL), showing the potential accuracy and utility of such an approach. In contrast to this *in silico* approach, Yan described a novel stable-isotope trapping, neutral-loss mass spectrometry (MS)–MS method to increase the reliability and sensitivity of reactive metabolite detection. Finally, Graham presented a custom built system used within her laboratory to identify metabolites. The combination of several analytical devices in an analysis module enables the rapid measurement of several parameters, facilitating the speedy, economical identification of metabolites.

Use of molecular tools to build metabolic screens

A touch of star-gazing was also incorporated into one of the sessions: where are we now in the *in vitro* analysis field and where should we be going? Brian Carr (Merck) began by looking at the use of CYPs expressed *in vitro* to examine drug metabolism. A large number of CYPs from several species are now available in Baculovirus expression systems, and Carr showed how these can be used to compliment the current 'traditional' systems. Of particular note is the use of Baculovirus-expressed primate CYPs to act as a bridge between rodent and human data. Next, Jonathan Dordick (Rensselaer Polytechnic Institute, NY, USA) introduced the MetaChip, a high-throughput cell-based screening technology on the micro-scale. This system enables rapid screening for drug toxicity and appears to be a highly exciting innovation.

Presentations by Douglas Conklin (University of Albany, NY) and Nick Plant (University of Surrey, UK) addressed two molecular approaches to improve understanding of the overall response to drug exposure. Conklin is

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using the extremely powerful technology of RNA interference (RNAi) to examine the role of individual proteins in cellular functioning, and reported the production of an RNAi library to knockout all known human mRNA transcripts (to date, approximately a third of the project has been completed). The use of such technology enables us to increase our knowledge of biological processes and identify novel therapeutic targets. Plant discussed the need to think of the cell as a whole and not just a series of independent processes, commenting that the way in which each process interacts with others is central to determining the overall response to a drug; these interactions must be understood before responses can be accurately predicted. The interaction of nuclear receptors in determining drug-mediated increases in CYP expression was used to illustrate this argument.

Conclusion

Conferences on *in vitro* technologies are common, but the data presented at this meeting showed why – the rapid progress in this field requires constant updating of the scientist to keep them abreast of the field, from the validation of cell systems to the development of cell toxicity microchips, which are the weapons in the arsenal of researchers. Although I feel that we are not yet in a position to extrapolate rapidly, and accurately, from *in vitro* to *in vivo*, we are at least moving towards the elusive crystal ball.

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Ligand efficiency indices as guideposts for drug discovery

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Successful drug discovery involves the optimization of many variables, such as compound potency, selectivity, cellular activity, solubility, metabolic stability, bioavailability and acceptable toxicity. It is a tortuous path beginning with lead selection and continuing through to preclinical testing in animal models. Efficient navigation through this hyper-variable space should be possible by reducing the number of variables to expedite the optimization process from lead discovery to evaluation in the clinic. Recently, the concept of ligand efficiency as a measure for lead selection was suggested. Here, a more comprehensive analysis of ligand efficiency indices is presented, including the introduction of three new indices: percentage efficiency; binding efficiency; and surface efficiency. These indices reduce the number of variables by combining potency with molecular weight and polar surface area. It is suggested that these indices, either individually or in combination, are useful markers for effective and efficient drug discovery, and might provide the basis for a mathematically robust optimization of the drug discovery process.

The complexity of the drug discovery process is well recognized. Crucial issues along the discovery path are lead selection and validation, followed by optimization strategies to achieve high potency and specificity at later stages. Discovery and optimization strategies often include structure-based technologies. An increasing volume of chemical, biochemical and clinical data support the concept that the intrinsic physicochemical parameters of putative pharmacological entities play a crucial role in their pharmacokinetic (PK) properties and therefore in their ultimate success as marketed drugs [1–3]. Wenlock *et al.* [3] documented a consistent increase in molecular weight (MW) at the clinical candidate stage, which was subsequently found to be counter-balanced by a trend towards lower MW and more acceptable pharmacological entities in marketable and successful drugs [3,4]. Other methods designed to aid in the identification of drugs from organic molecules have been considered, including: characterization of molecular scaffolds and substituents [5,6]; statistical analysis of different drug databases [7]; the use of neural networks [8–10]; analysis of property

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