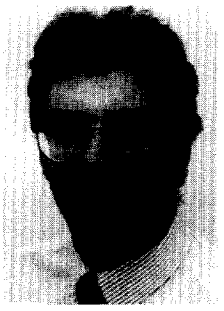
Modifying the drug discovery/ drug development paradigm



How do we improve the selection of candidate compounds?

The advent of combinatorial chemistry and the development of high-throughput screening (HTS) techniques for the identification and evaluation of potential lead compounds will undoubtedly result in an increase in the number of potential drug candidates moving from discovery into development. With only 0.1% of preclinical compounds reaching human clinical trials following, in many cases, extensive development and associated resource investment, there is a clear case for reviewing the drug discovery/development paradigm in order to improve the selection of candidate compounds.

The three main reasons why preclinical compounds are rejected during drug development are intrinsic toxicity, metabolite toxicity or poor bioavailability. These properties are traditionally determined from efficacy and toxicity studies in animals and are time-consuming, relatively expensive and may reflect the *in vivo* situation in man inadequately. Recent advances in molecular and cell biology have provided opportunities to develop reflective *in vitro* models to assess these factors more rapidly as part of the drug development programme.

In vitro evaluation of drug metabolism

Is it possible to undertake some metabolic evaluation studies as part of the drug discovery screening programmes? Clearly, the use of animal models at this pre-development stage is inappropriate because of prohibitive costs and associated ethical issues; we must therefore look towards various *in vitro* models as potential alternatives. Cashman has described the use of human cDNA-expressed enzymes, microsomes, hepatocytes and tissue slices as an alternative approach to traditional animal-based metabolism studies[1]. Recent studies using these systems have clearly demonstrated that such models may be used to obtain *in vitro* drug metabolism data that may be used to effectively predict *in vivo* parameters. A limiting factor in the use of this technology may be the availability of the enzyme and/or human tissue for large-scale screening.

Microbial models of drug metabolism

An alternative approach, which has been investigated by various groups over the past 20 years, is the possible utilization of microbial models of drug metabolism[2–6]. Numerous studies have now shown that both the Phase I and Phase II metabolic biotransformations seen in mammalian systems are paralleled in microorganisms. Phase I metabolism includes primarily oxidation, reduction, hydrolysis and hydration reactions, whereas phase II metabolism involves processes leading to the conjugation of drugs to various moieties, generally resulting in water-soluble products that can be excreted in the bile or urine. Phase II processes include glucuronidation, glycosidation, sulphonation, methylation, acetylation, amino acid conjugation, glutathione conjugation and fatty acid conjugation. Of particular interest are the diverse Phase I mammalian oxidations associated with the microsomal mixed-function cytochrome P450-dependent oxidase system. These include the processes of aromatic hydroxylation, aliphatic hydroxylation, epoxidation, dealkylation, oxidative deamination and dehalogenation.

Various microorganisms have been shown to possess cytochrome P450 monooxygenase enzyme systems that mimic the mammalian microsomal mixed-function cytochrome P450-dependent oxidase system. Prokaryotic (bacterial) P450 monooxygenase enzyme systems appear to display a very narrow substrate specificity resembling extrahepatic cytochrome P450 systems, whereas the P450 systems in eukaryotes such as fungi are broader in their substrate specificity. The fungal monooxygenases appear to be involved in a wider range of metabolic processes, thereby offering potential as effective models of mammalian P450 oxidation processes. Microbial models offer the potential advantages that microorganisms are more readily cultured than are mammalian cells, they are less susceptible to environmental factors such as temperature, pH, aeration and media composition than are mammalian cells and may be readily scaled up to allow isolation of metabolites for structure elucidation or for use as analytical standards in subsequent mammalian studies.

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Drug absorption evaluation using cell culture models

Cell culture models of drug absorption may be used to gain useful information on drug permeability and mechanism of transport across epithelial barriers and may indicate likely bioavailability. The importance of such preclinical studies has been highlighted by the routine use of the well characterized Caco-2 cell model to screen compounds for potential oral bioavailability. Major pharmaceutical companies have either established in-house facilities or are outsourcing to the increasing number of contract research organizations specializing in this field.

Cell lines have also been developed as potential models for the assessment of transdermal, nasal, pulmonary and vaginal drug absorption, enabling the assessment of the potential bioavailability of drug candidates by different routes of administration. Such model systems have important application at the preclinical stage as a means of assessing *in vitro* the effects of drug formulation on drug absorption. Furthermore, issues of drug toxicity and complications such as P-glycoprotein efflux may be readily assessed using such systems. The major disadvantage of existing cell culture models is the time involved in culturing confluent monolayers of cells for such studies, which can exceed 21 days for some cell lines. Such systems have also been criticized because the cell lines used are not truly reflective of the *in vivo* situation. For example, confluent monolayers of Caco-2 cells are less leaky than the gastrointestinal epithelial cells in the small intestine and do not have an associated mucus layer, which represents an additional barrier to many drugs. In spite of these limitations, it is now generally accepted that such models are useful for the identification of compounds that are likely to have good bioavailability, for the direct comparison of compounds from a similar class in order to establish the structural properties affecting drug absorption and for the evaluation of the effects of formulation parameters, such as excipient mixtures, on drug absorption.

Although intrinsic toxicity may be detected during cell-based HTS, information on metabolic toxicity and potential bioavailability tends to be addressed early in the development programme. With the recent developments in bioanalytical techniques such as LC-MS, which offer the potential to analyse aqueous solutions for absorbed drug and/or metabolites readily, such assays may be relatively easily incorporated into HTS drug discovery programmes. The incorporation of *in vitro* drug absorption and metabolism assays into HTS drug discovery programmes will benefit the lead optimization programmes and ensure that the best candidates are promoted into development. Elimination of untenable compounds at this early stage would also reduce unnecessary synthetic development and scale-up costs.

Existing *in vitro* models of drug metabolism and absorption clearly offer potential for the initial pre-development screening of candidate compounds in order to select compounds with appropriate metabolic and absorption profiles for further evaluation. Further work in these fields will undoubtedly provide more efficient and reflective *in vitro* models to aid the selection of suitable candidate molecules for pharmaceutical development from the increasing number of new chemical entities emerging from drug discovery programmes as a consequence of the revolution of drug discovery caused by the introduction of combinatorial chemistry techniques.

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