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Use of human tissue in ADME and safety profiling of development candidates

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The clinical success of a compound is often curtailed because of inadequate safety, pharmacokinetics or efficacy. Human tissue can be used to identify the potential shortcomings of new drugs before they undergo testing in man. This review highlights the consent and ethical approval required for the use of human tissues and discusses their use for predicting human ADME and safety profiles of drugs in preclinical development. The ability to retrieve a wide range of viable tissues from human donors provides the opportunity to test drugs for many potential use-limiting side-effects.

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▼ The process of bringing new drugs to the market is a remarkably inefficient one, with >90% of the drugs that enter the clinical phase of development failing for reasons of inadequate safety, pharmacokinetics (PK) or efficacy. One important reason for such a poor success rate relates to the excessive reliance placed on data obtained from animal experiments and recombinant systems in both the discovery and development phases of the process. Because there are so many well-documented inter-species differences in physiology and pathology, and the principal target of the pharmaceutical industry is man, it is not surprising that non-human species often prove to be unreliable surrogates in drug development programmes and can provide misleading information. It could be argued, therefore, that an understanding of the effects of new drugs in human tissue would be extremely useful in determining the relevance of experimental data derived in other species during preclinical development. It is increasingly common for human tissue to be used in establishing safety, PK and efficacy profiles of development candidates before testing in humans. Although it is not yet a requirement of the regulatory authorities to incorporate such data in drug submissions, it seems increasingly probable that it will be in the future. Some such uses, for predicting the drug absorption, metabolism, drug–drug interactions, cellular toxicity and safety pharmacology of new drugs for clinical use are described.

Acquisition of human tissue

Human tissue can be acquired for pharmaceutical research use, provided that ethical and legal guidelines have been rigorously adhered to. Paramount among these are the written and informed consent of the donor and/or the donor's legal representative, guaranteed donor anonymity and approval of the supply of the tissue for the stated purpose by an independent ethical review committee. The guidelines covering such use are outlined in several documents, including those from the Nuffield Council Report[1], the Medical Research Council[2] and the Royal College of Pathologists[3]. In the acquisition of these tissues, it is essential that procedures are in place that permit the retrieval and subsequent transfer to the laboratory as rapidly as possible, under conditions ensuring optimal viability. It might also be necessary for tissues to receive specific treatment within the operating theatre immediately upon removal from the body and, to ensure this, there must be a strong collaborative relationship between hospital and researcher.

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Drug absorption

A key characteristic defining the oral bioavailability of a drug is the ease with which the drug is absorbed by the gastrointestinal (GI) tract. Several models of drug absorption have been applied to predict absorption in the human gut. These include *in vivo* studies with animals and *in vitro* studies using cell lines, predominantly Caco-2 (reviewed in Refs 4,5). Caco-2 monolayers have been used to rank the permeabilities of many structurally diverse compounds, and good correlations between absorption and Caco-2 permeability were demonstrated[6,7]. However, in general, the ability of such models to predict absorption in the clinic is somewhat variable. For example, compared with the drug diazepam, both verapamil (a calcium-ion channel blocker) and 5-fluorouracil (an antimetabolite) have lower human bioavailabilities than might be predicted from their permeabilities in rabbit ileum[8], whereas Caco-2 cells often demonstrate low rates of transport[5] and could underestimate absorption resulting from the over expression of P-glycoprotein (P-gp; Ref. 7).

The majority of drugs are absorbed in the higher gut (duodenum, jejunum and ileum) and so there have always been some questions as to how well colon-derived cell lines, such as Caco-2, can model human absorption. In addition, differences in drug permeability *in vivo*, between model systems and humans, are likely to be partly a result of the activity of active drug transporters that modulate drug uptake and, hence, passage through the epithelial cells lining the GI tract. The most well-characterized of these is the multidrug resistance transporter (MDR), or P-gp, which is located on the mucosal surface of the GI epithelial cells. Drugs that are substrates for P-gp can be extruded from cells via an energy-dependent process after they have entered via other routes. Recently, transformed cell lines, such as LLC-PK1 and MDCK, expressing P-gp have been developed and are now being used to screen compounds for transporter substrate specificity[9,10]. However, several other transporters that are able to import or export organic anions and cations have been identified, and it is probable that these also have important roles in regulating the movement of drug from the GI tract into the blood stream[11]. The use of human cells to study the influence of such transporters is important because there is clear evidence for significant species differences in both their expression and their function[12–14]. So, although cell-line systems are useful as high-throughput screens to eliminate early-stage compounds with too low a passive permeability to be orally absorbed, caution is advised for compounds whose GI uptake is determined mainly by the action of transporters[15] because these cell-line models lack equivalence to the native tissue.

To reduce such risks, drug transport can be studied using the mucosal layer isolated from appropriate regions of freshly isolated human gut obtained from GI surgery. A major advantage of using intact human GI mucosa is that the native tissue will contain the appropriate variety and expression of transporters that are found *in vivo*. In addition, tissue from along the length of the GI tract could be sourced, allowing a comparison of absorption in different areas of the gut. One potential issue with using fresh human gut tissue could be retaining the function of these transporters during the time taken to transport the tissue to the laboratory. This again highlights the importance of good relationships with tissue suppliers to ensure that the human GI tissues arrive in a healthy, viable state.

Drug absorption across human gut mucosa can be studied using the Ussing chamber[16]. A section of mucosa can be sandwiched between a pair of chambers each perfused with oxygenated physiological buffer at 37°C. Drug is added to one chamber and the transport between chambers is determined from measurements of drug in samples taken from both chambers at regular intervals. Transepithelial electrical resistance can be monitored throughout the experiment as an indicator of mucosal viability. Figure 1 shows an example of an experiment in which the transport of propranolol has been measured across the human colonic mucosa. Low transepithelial conductance and rate of transport of the non-absorbable marker [^{14}C] PEG4000 throughout the experiment confirm the integrity of the epithelium, and the relatively large transfer of [^{3}H] propranolol from mucosal to serosal sides of the epithelium is consistent with the known high absorption of propranolol in humans. The mean apparent permeability (P*app*) for propranolol absorption across human colonic mucosa was calculated to be 100.1×10^{6} cm sec^{-1}. This is approximately threefold greater than the P*app* for propranolol calculated using Caco-2 cells[6,7]. These data support the view that Caco-2 cells might underestimate rates of transport[5].

The discovery of human drug transporters, their expression and polymorphism is a growing area of research. Expression of the variety of different transporters that might be involved in drug transport can be measured using techniques such as quantitative reverse-transcriptase PCR (qRT-PCR). This can be performed using small sections of the same samples of gut mucosa used for drug absorption experiments and could help with data interpretation. In addition to the use of fresh human tissue for studying drug absorption, tissues from the human GI tract will prove invaluable to identify novel transporters and gain insight into the inter-individual expression of drug transporters and the impact of their expression on oral bioavailability.

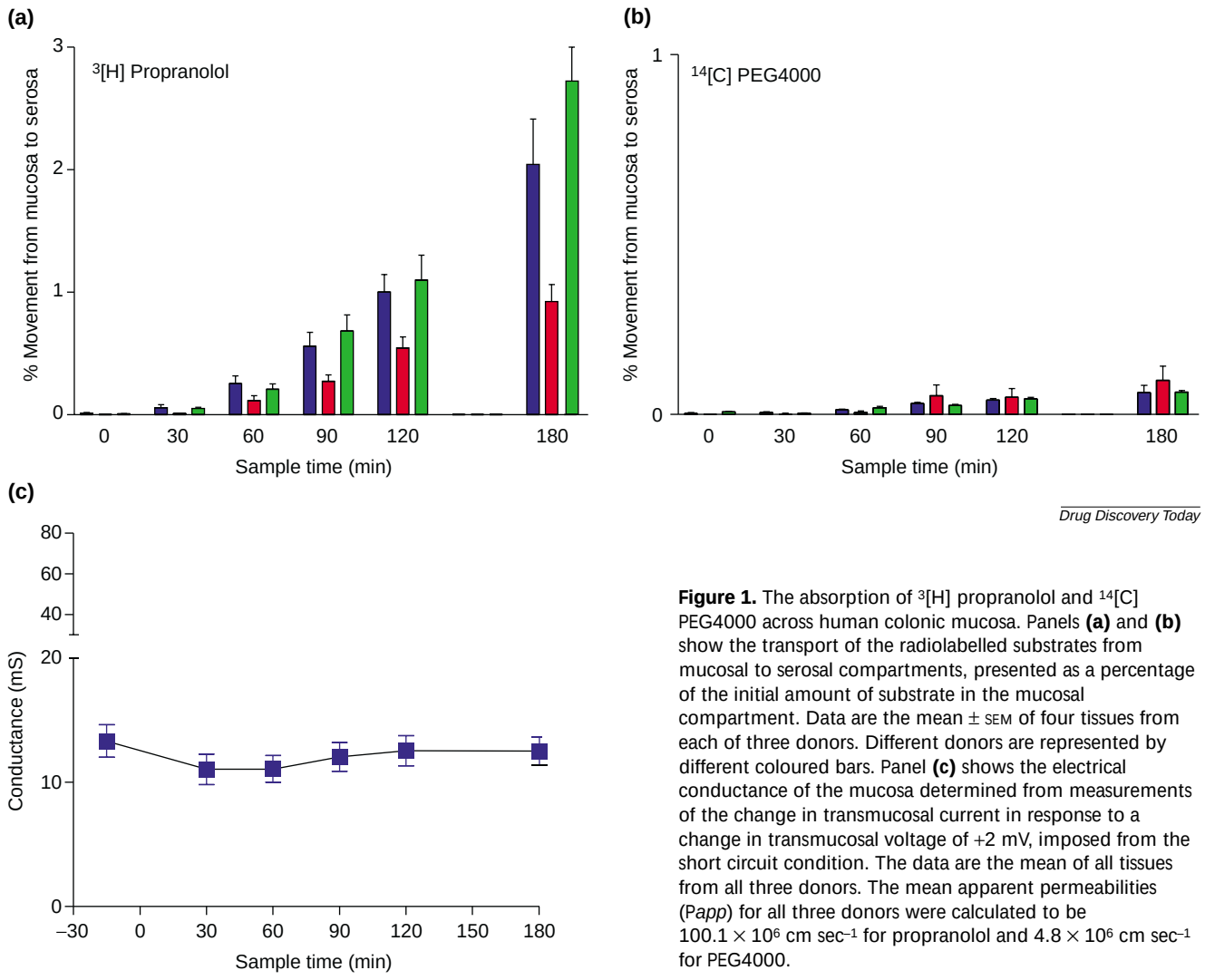

Figure 1. The absorption of ^{3}[H] propranolol and ^{14}[C] PEG4000 across human colonic mucosa. Panels **(a)** and **(b)** show the transport of the radiolabelled substrates from mucosal to serosal compartments, presented as a percentage of the initial amount of substrate in the mucosal compartment. Data are the mean ± SEM of four tissues from each of three donors. Different donors are represented by different coloured bars. Panel **(c)** shows the electrical conductance of the mucosa determined from measurements of the change in transmucosal current in response to a change in transmucosal voltage of +2 mV, imposed from the short circuit condition. The data are the mean of all tissues from all three donors. The mean apparent permeabilities (P*app*) for all three donors were calculated to be 100.1×10^{6} cm sec^{-1} for propranolol and 4.8×10^{6} cm sec^{-1} for PEG4000.

Drug metabolism

Metabolism is a key determinant of how a drug ultimately performs *in vivo*. Classically, this has been evaluated preclinically *in vivo* and *in vitro* in non-human species, typically one rodent and one non-rodent species. However, there are many examples of xenobiotics whose routes or rates of metabolism vary between species[17–20] making extrapolation to man hazardous. Such variation might arise from inter-species differences in the substrate specificity of enzymes responsible for metabolism or from variation in the levels of expression of drug-metabolizing enzymes.

Human liver cell lines, such as HepG2, offer little help, because in many important respects they are unrepresentative of the organs from which they were originally derived. However, there is one clone of HepG2, C3A, which has been demonstrated to possess activity for CYP1A1 and CYP3A4 (Refs 21,22).

Human liver tissue

Fortunately, drug metabolism in humans can now be investigated *in vitro* using human tissue. Many organs in the body contribute to the metabolism of administered drugs, but the most important is undoubtedly the liver. Various *in vitro* preparations of human liver can be used, including microsomes, cytoplasmic fractions, S9 fractions, liver slices and isolated hepatocytes. The use of human liver subcellular fractions and isolated human hepatocytes for studying the metabolic stability of new chemical entities (NCEs) has recently formed part of a review in this journal[23]. Although each of these preparations has its uses, the freshly isolated hepatocyte is widely regarded as the gold standard for predicting hepatic drug clearance and the route of metabolism in humans. Only intact hepatocytes provide all the components of drug metabolism pathways (enzymes and cofactors) in the relevant concentrations, environment and cellular compartmentalization.

Isolation of viable human hepatocytes requires considerable experience, as well as rapid access to surgically excised liver to prepare cultures suitable for drug metabolism studies. Although, in our experience, access to human liver tissue for hepatocyte isolation is improving, this is often a rate-limiting factor for the routine study of drug metabolism. With this in mind, there have been recent improvements in methods for reliable cryopreservation of human hepatocytes[24]. Cryopreserved human hepatocytes now retain almost all of their drug metabolizing capabilities and can be used to study metabolic stability and to determine rates and routes of metabolism of an NCE.

Viability of hepatocytes is usually measured by their ability to exclude Trypan Blue dye, to metabolize a range of standard compounds and to adhere to collagen-coated plates in culture. In suspension, hepatocytes remain viable for a limited time (up to six hours) but they can be maintained in adherent culture for up to several weeks[25] and used to study drug metabolism, drug–drug interactions and toxicity. Although human hepatocytes can be cryopreserved, thawed cells rarely adhere to collagen-coated culture plates[24] and, as such, are not suitable for longer term studies in monolayer culture.

To study the rate of hepatic drug metabolism *in vitro*, drugs can be incubated with human hepatocytes in suspension, and the levels of parent drug and metabolite can be determined at intervals by HPLC or LC–MS. Studies of metabolic route may require longer incubations (greater than six hours) and in this case a monolayer culture of human hepatocytes can be used. However, it is important to consider the decline in expression of drug metabolizing enzymes in hepatocytes placed into conventional monolayer culture and, hence, drug metabolism studies performed in monolayer culture should be regarded as qualitative and not quantitative.

Many different enzymes contribute to drug metabolism but the major enzymes involved are the cytochromes P450 (CYPs). In humans, the most significant of these are the CYP3A family, CYP2D6, CYP2C9 and CYP1A2, which, between them, are involved in the metabolism of two-thirds of drugs tested[26]. For each individual isolation, the activities of these key enzymes in the hepatocytes should be confirmed using specific substrates for these enzymes (Table 1). The enzymes responsible for drug metabolism are subject to inter-individual variability; indeed several of the CYPs are polymorphic[27] (www.imm.ki.se/CYPalleles/). Therefore, variation in CYP expression and function can result in a misleading indication of the metabolism of an NCE if the drug is studied in hepatocytes from a single donor. However, by measuring the catalytic activities of the major drug metabolizing enzymes in human hepatocytes, key differences in catalytic activity between donors can be determined. In addition, the genotype of the donor can be important for interpreting any data obtained.

Table 1. Substrates suitable for studying the activity of specific cytochromes P450 (CYP) in human hepatocytes

Human CYP	Substrate	Major metabolite
1A2	Caffeine 7-Ethoxyresorufin	Paraxanthine Resorufin
2A6	Coumarin	7-OH-Coumarin
2B6	S-Mephenytoin	Nirvanol
2C9	Diclofenac	4-OH-Diclofenac
2C19	S-Mephenytoin	4-OH-Mephenytoin
2D6	Bufuralol Debrisoquine	1-OH-Bufuralol 4-OH-Debrisoquine
2E1	Chlorzoxazone	6-OH-Chlorzoxazone
3A4	Testosterone Midazolam	6β-OH-Testosterone 1-OH-Midazolam

Membrane transport activities, such as hepatic uptake and biliary excretion, also have a role in the hepatic clearance of drugs. Hepatocytes can be used to study the effect of drug transporters, such as P-gp and organic-ion transporters[28], whose characteristics and activity both in non-human hepatocytes and in liver cell-lines, such as HepG2, are different from those in the native human tissue[29,30]. Short-term cultures of freshly isolated hepatocytes can be used to measure clearance by hepatic uptake. These experiments should be performed as soon after hepatocyte isolation as possible because prolonged culture downregulates some cell membrane transporters[31].

Extrahepatic metabolism can also have an important role in drug clearance. Human tissues that are important in extrahepatic metabolism, such as the GI tract, kidney and skin, can also be used to provide information during the preclinical phase of drug development. Although cells, tissue slices or subcellular fractions are generally used to determine the extrahepatic metabolism, sections of GI mucosa mounted in Ussing chambers to measure drug transport can also be used to assess gut metabolism.

Prediction of drug–drug interaction

A cause for concern with any NCE introduced into the body is the influence it might have on its own metabolism, as well as on that of any co-administered drugs. A drug might inhibit metabolic enzymes, an effect that can be studied using hepatocyte suspension culture[23], but it might

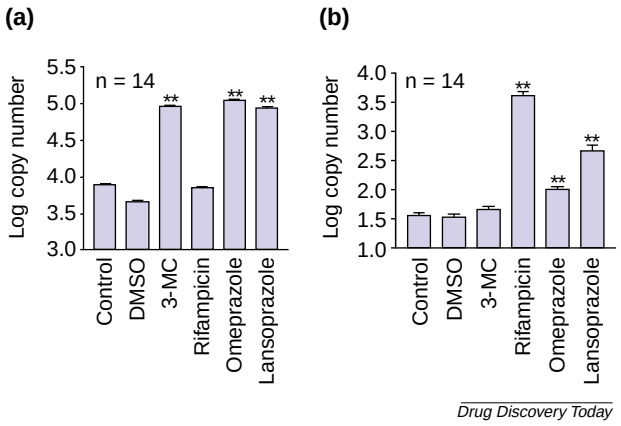

Figure 2. Induction of mRNA encoding **(a)** CYP1A1 and **(b)** CYP3A4 in isolated human hepatocytes exposed to rifampicin (50 μM), 3-methylcholanthrene (3-MC; 1 μM), omeprazole (50 μM) and lansoprazole (50 μM). Human hepatocytes were treated for 48 hours with the test compounds. Total RNA was extracted and quantitative reverse-transcriptase-PCR performed with primer and probes specific for CYP1A1 and CYP3A4. The data represent the mean mRNA abundance (Log copy number ± SEM) in 100 ng of total RNA from a total of 14 donors.

also induce the production of those enzymes. Enzyme induction can result either in a marked increase in production of a toxic metabolite[32,33] or in an increased rate of elimination of co-administered drugs, with a concomitant reduction in their efficacy. For example, phenobarbital induces CYP2B6 and CYP3A4, increasing the reductive dehalogenation of halothane, thus reducing its effectiveness and increasing the production of toxic metabolites leading to halothane-induced liver injury[34,35]. The anti-tuberculosis drug, rifampicin, is known to induce CYP3A4 with well-described effects of reducing the effectiveness of some oral contraceptives[36,37]. Similarly, hyperforin, a component of the anti-depressant herbal remedy, St John's Wort, also induces CYP3A4, and can lead to reduced efficacy of a large number of clinically prescribed drugs[38]. Herbal preparations, such as St John's Wort, and foodstuffs, such as grapefruit juice, can also contain chemicals that interact with metabolic enzymes resulting in competition with drugs for metabolism by these same enzymes[39,40]. Like drug metabolism itself, such effects can also be species-dependent. For example, dexamethasone is a potent inducer of CYP3A1 in the rat but is a poor inducer in humans, whereas omeprazole is a potent CYP1A1 and CYP1A2 inducer in humans, rabbits and dogs but not in rats[41,42]. This highlights the value of early testing of potential drugs in human tissue wherever possible.

In addition to understanding the metabolism of a new drug, it is important to establish whether it is likely to affect the metabolism of other co-administered therapies. To study the effects of a drug on hepatic gene expression, longer-term monolayer culture of human hepatocytes in a 96-well format can be used. Three days after isolation, when the expression of genes encoding metabolic enzymes has stabilized, cells are exposed to drugs of interest and the expression of messenger RNA (mRNA) for drug metabolizing enzymes is determined. Total RNA can be extracted robotically, each well providing sufficient mRNA to quantify the expression of up to 100 different gene transcripts using qRT-PCR (Ref. 43).

Messenger RNA represents a useful marker of induction because enzyme-inducing agents appear to act largely at the level of transcription through interaction with specific upstream response elements[44,45]. Quantification of mRNA using qRT-PCR, based on TaqMan® technology and the ABI Prism 7700 Sequence Detection System (Applied Biosystems, Warrington, UK), provides a highly robust, reproducible and sensitive approach to mRNA detection, and is sensitive to about ten copies in 100 ng total RNA (equivalent to ~50,000 cells). This is not an endpoint assay but involves real-time determinations, enabling the relationship between PCR cycle and product formation to be monitored continuously. Quantification of mRNA expression is possible without PCR substrate depletion being a problem.

To validate this method, the effects of known inducers have been studied[37]. For example, 3-methylcholanthrene, omeprazole and lansoprazole, which are known inducers of CYP1A1, produce an approximate tenfold increase in the expression of the mRNA for this enzyme, whereas rifampicin, which does not induce CYP1A1, does not cause this effect. By contrast, rifampicin does induce CYP3A4, producing a 100-fold increase in specific mRNA expression, whereas 3-methylcholanthrene is ineffective (Fig. 2). The ability to determine multiple genes in each well makes it possible to determine the effects of a compound on all of the known CYPs in the same cells.

Drug treatment can also result in the induction of enzymes other than the CYPs, including alcohol- and aldehyde-dehydrogenases, glutathione *S*-transferases, sulfotransferases and glucuronyl transferases. Using qRT-PCR, it is possible to study changes in expression of phase I and phase II enzymes, together with a range of other genes associated with ADME, such as nuclear receptors and transporters. So, from a single 96-well experiment, a large amount of information can be obtained and compared with corresponding data obtained with reference drugs, such as rifampicin and omeprazole. The use of different concentrations of inducing agent demonstrates that induction can not only be quantitatively substantial but also concentration-related.

Table 2. Bioactivation of xenobiotics; the metabolism of compounds to form toxic or reactive intermediates

Mechanism	Description	Example	Mediated via	Toxic product	Associated toxicities
I	Biotransformation to stable, toxic metabolites	Dichloromethane	Cytochrome P450	Carbon monoxide	
II	Biotransformation to reactive, electrophilic intermediates	Paracetamol	Cytochrome P450	*N*-acetyl-*p*-benzoquinone-imine	Protein alkylation Glutathione depletion Alkane expiration Increased cytosolic calcium Cytoskeletal changes Protease activation Lipase activation
III	Biotransformation to free radicals	Carbon tetrachloride (CCl_4)	Cytochrome P450	$.CCl_3$	Lipid peroxidation Inhibition of protein synthesis Covalent binding to cellular lipids Altered intracellular calcium homeostasis
IV	Formation of reduced oxygen metabolites (more common in extrahepatic tissues, but has been extensively studied in isolated hepatocytes)	Menadione	NADPH cytochrome P450 reductase	Oxidation back to the quinone yields superoxide anion radical	Oxidative stress Enhanced by glutathione depletion Oxidation of purine nucleotides Thiol oxidation/arylation Altered intracellular calcium homeostasis
V	Metabolic derangements associated with xenobiotic transformation	Galactosamine	Hepatic uridine triphosphate deficiency	No reactive intermediate formed	Altered intracelluar calcium homeostasis
		Fluoroacetate		Fluorocitrate	Inhibition of aconitase and subsequent perturbation of Krebs cycle

Molecular toxicology

As part of the drug discovery process, assessment of toxic potential is used to prioritize compounds for further evaluation. By assessing a probable safe dose to administer during clinical development and evaluating risk caused by prolonged exposure, resources can be saved by focussing on compounds that are more likely to succeed. Drug toxicity is conventionally determined in two animal species by examining a variety of changes, which include serum-chemistry parameters and histopathology. Human risk is then assessed by extrapolation from the results of animal studies. However, toxicity might reside in the parent drug or its metabolites and, therefore, any test system should evaluate the effects of not only the drug itself but also its metabolic products (Table 2). The importance of knowing the metabolites produced in humans is paramount for assessing human drug safety.

Human in vitro *toxicology*

Human *in vitro* cell systems have been developed to provide information on drug and chemical toxicity. The most frequently used systems involve cells or tissues from liver, kidney, eye or skin. These models can provide valuable information for safety assessment through mechanistic studies, comparative toxicity in animal- and human-derived cell systems and the provision of data allowing estimates of safe exposure margins or probable toxic dose levels in humans. Recently, the status of human *in vitro* models used for risk assessment has been reviewed and recommendations made for the use and improvement of these models[31].

A key example of the potential use of human-based toxicity systems is illustrated by the quinoxalinone anxiolytic, panadiplon, which was discontinued from clinical development because of its unexpected hepatic toxicity in human volunteers. No evidence of toxicity had been found in preclinical development studies in dogs, rats or monkeys. However, subsequent studies conducted in rabbit and human hepatocytes demonstrated the disruption of certain mitochondrial activities by panadiplon and identified a carboxylic acid metabolite, which might be involved in toxicity associated with panadiplon[46].

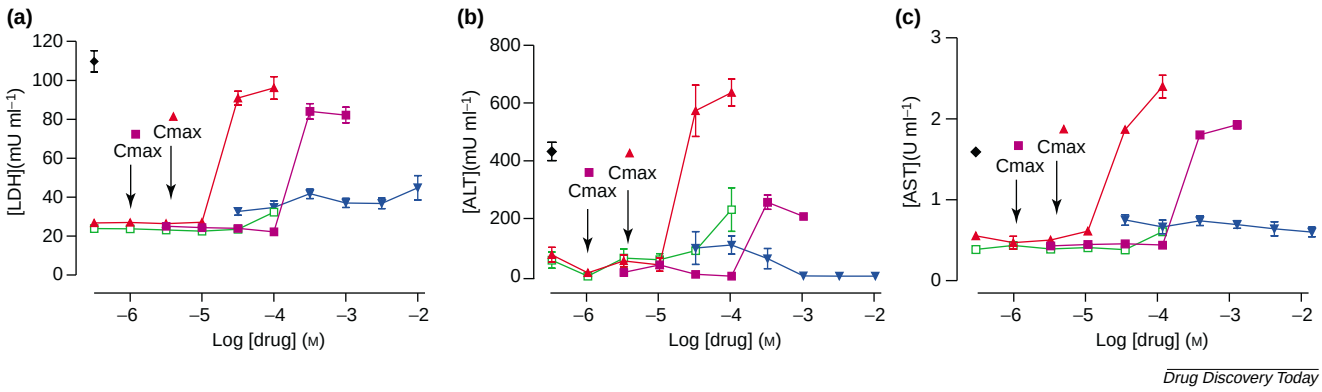

Figure 3. The effect of troglitazone (▲), rosiglitazone (■), pioglitazone (□), acetylsalicylic acid (▼) and 1% Triton X-100 (◆) on (a) lactate dehydrogenase (LDH); **(b)** alanine aminotransferase (ALT) and **(c)** aspartate transminase (AST) release from human hepatocyte monolayers treated for 24 hours with test compounds. Data are the mean ± SEM and are representative of three donors. The arrows indicate the equivalent clinical plasma concentrations for troglitazone (▲) and rosiglitazone (■). In all cases, the apparent *in vitro* toxicity of troglitazone occurs approximately half a log unit from the clinical plasma concentration, whereas for rosiglitazone, the apparent *in vitro* toxicity occurs approximately two log units from the clinical plasma concentration resulting in a much larger safety window.

The liver is the principal metabolic site for most drugs and, therefore, an initial assessment of toxicity in isolated hepatocytes is often performed. An overview of biochemical assays commonly used to study hepatotoxicity has been presented in an earlier review in *Drug Discovery Today*[23]. Recently, the thiazolidone antidiabetic compound, troglitazone, has been withdrawn from the market because of an observation of idiosyncratic hepatotoxicity following clinical use. Clinical signs included increased levels of the liver enzymes alanine aminotransferase (ALT) and aspartate transaminase (AST). The question facing the pharmaceutical industry is whether the effects of troglitazone are peculiar to that compound, or are mechanism-related. Tests on cultured human hepatocytes using troglitazone have revealed that it causes several toxicity-related effects at high concentrations, including the release of lactate dehydrogenase (LDH), AST and ALT and decreased protein synthesis[47,48]. Further studies comparing the ability of troglitazone to induce these effects with those of a competitor thiazolidone, rosiglitazone, revealed that although rosiglitazone also had some hepatotoxic potential, these effects were only produced at tenfold higher concentrations[47]. Although it is not certain that these *in vitro* effects relate directly to the idiosyncratic hepatotoxicity reported with troglitazone, this finding, together with the lower clinical doses required for rosiglitazone compared with troglitazone, suggests that rosiglitazone would have a potentially greater safety margin than troglitazone (Fig. 3).

Human *in vitro* models have been developed for extrahepatic sites of toxicity, such as the eye, skin and kidney. Ocular toxicity can be assessed using models ranging from monolayer cultures of human keratinocytes and corneal epithelial cells for acute studies, three-dimensional (3D) models of corneal epithelium to assess chronic effects of agents, such as preservatives for contact lens preparations, to artificial corneas constructed entirely from human cells[31]. Similarly, human skin irritation models vary in complexity from monolayer cultures of human keratinocytes or fibroblasts, to 3D models of the human epidermis. The former are used to test the potential toxicity of individual ingredients on specific cells, whereas the latter are used to test mixtures and final formulations. Such models are commercially available, for example the EpiDerm™ human skin and EpiOcular™ human eye models (MatTek Corp., Ashland, MA, USA).

Nephrotoxicity can be assessed using freshly isolated glomeruli and nephron fragments, although their life span is limited to only a few hours, thereby restricting their use to acute studies[49]. An alternative is monolayer cultures of human glomerular and proximal tubule cells; proximal tubule cells have been used to study the potential nephrotoxicity of several xenobiotics, including the aminoglycoside antibiotics, gentamicin, neomycin, kanamycin and streptomycin[50].

Human lung, heart, intestine and lymphoid tissue can also be used to investigate toxicity. In fact, almost any human tissue can be used to study basic cytotoxicity.

Toxicogenomics

Cellular toxicity can manifest itself in a wide variety of different forms, including necrosis, apoptosis, inflammation, cholestasis, fibrosis and/or cirrhosis and carcinogenicity. With this wide range of toxic mechanisms and outcomes, many *in vitro* models have focussed on fairly general

biochemical or functional markers of cell toxicity. To further elucidate the mechanisms of toxicity, functional assays can be combined with gene expression assays that measure changes in key pathways involved in toxicity.

Sequencing of the human genome has led to the advent of toxicogenomics, which uses DNA microarrays and bioinformatics to identify potential gene markers of toxicity[51] by detecting genes whose expression is modulated following drug treatment. This approach can increase our understanding of mechanisms of toxicity or provide early predictions about the adverse effects of a compound. The use of applied microarray technology to study human toxicity involves the treatment of cells with compounds grouped by class, target, mechanism, structure or toxic endpoint, to create specific patterns of gene expression changes. These cells can be isolated from a variety of human tissues. Such studies are leading to the development of databases of 'fingerprint responses' to aid the prediction of human *in vivo* responses by comparison of human *in vitro* responses with those obtained from animal studies[31].

Toxicoproteomics

More recently, proteomics has emerged as a tool for the identification of toxicity markers to complement genomics-based approaches. By studying the inter-relationships between proteins following drug treatment, the mechanistic basis for drug toxicity can be determined[52]. The primary tool used to achieve this is 2D gel electrophoresis, which separates thousands of individual proteins in a given sample. This enables the detection of differentially expressed proteins following, for example, exposure to xenobiotics[53]. Such studies will aid our understanding of the mechanisms of toxicity for specific xenobiotics, and permit the identification of protein markers for risk assessment and individual susceptibility factors at the protein and the gene level. With this in mind, proteomics may provide another valuable approach to establish protein change 'fingerprints' associated with different classes of toxicant.

Safety pharmacology

Finally, there are many pharmacological actions of new drugs that can limit their potential clinical use, including unwanted constrictor effects in the cerebral and coronary vasculature, effects on cardiac contractility and ocular lens opacification. Cerebral vascular tissue can be obtained rapidly after death and can be prepared for pharmacological evaluation. Using traditional organ-bath techniques, it is possible to test compounds for their abilities to either constrict or dilate cerebral vascular smooth muscle.

Viable cardiac tissue available from human hearts includes atrial appendage and coronary vasculature. The coronary vasculature can be examined in a similar fashion to the cerebral vasculature, to detect any possible constrictor or dilator effects. Atrial appendage can be used to prepare single atrial trabeculae; these structures beat spontaneously and so the direct effects of drugs on cardiac muscle contractility can be evaluated. The use of atrial trabeculae enables the quantification of both positive and negative effects on the force and rate of atrial beat.

A well established example of a drug exhibiting coronary side-effects is sumatriptan, a drug developed by Glaxo (Ware, UK) in the 1980s for the treatment of migraine. Migraine is believed to result from a vasodilatation of the cerebral vasculature, which causes pressure on sensory afferent nerves, leading to pain, nausea and the associated visual symptoms. Sumatriptan is thought to exert its anti-migraine effect, at least in part, through a direct constrictor action on the cerebral vasculature mediated through a sub-class of serotonin receptors (5-HT_{1B}). Although the drug is highly effective in the majority of migraine patients, its beneficial effects are counterbalanced by the incidence of chest pains in a substantial minority of patients. There is evidence that these pains result from a vasoconstrictor effect on the coronary vasculature. Subsequent studies have revealed that not only are 5-HT_{1B} receptors expressed in the cerebral vascular bed but also in the coronary vasculature, in many individuals. Whereas the density of this receptor population and the magnitude of the vasospastic effect observed following sumatriptan treatment show high degrees of inter-individual variation[54], the incidence of this unwanted side-effect is enough to cause concern. It is now clear that this effect could have been predicted by simple studies in human coronary vascular tissue (Fig. 4).

Another important potential cardiac side-effect is QT-interval prolongation. The QT interval of the electrocardiogram (ECG) corresponds to the time between depolarization and repolarization of ventricular myocardium during the cardiac action-potential[55]. A prolongation of this interval is characterized by polymorphic ventricular tachyarrhythmias, which can cause syncope (temporary loss of consciousness) or sudden death. Therefore, it is desirable to test for QT-interval prolongation at an early stage of drug development. The cardiac action-potential reflects the summation of voltage changes across the cellular membrane, which are generated by the influx and efflux of ions via voltage-gated ion channels, ion pumps and ionic exchangers. Current methods range from *in vivo* electrophysiology studies using animal species, to electrophysiology- and cell-based assays using heterologously expressed human

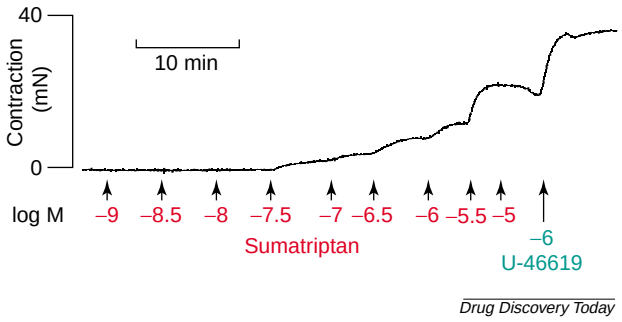

Figure 4. Contractile responses of an isolated ring preparation of human coronary artery to increasing concentrations of sumatriptan. The response to the thromboxane mimetic, U-46619, represents a maximal contractile response on this preparation.

ion channels in cultured cells. The disadvantages of the *in vivo* studies are high cost, low throughput and inter-species variation, whereas the *in vitro* studies fail to effectively model the *in vivo* phenomenon. Therefore, a combination of both approaches is usually required. The development of reliable human *in vitro* electrophysiology-based assays could provide a more relevant model and, therefore, aid the screening of compounds for their potential to induce QT-interval prolongation.

Another organ that is particularly sensitive to drug-induced toxicity is the eye. Many drugs can induce lens opacification – cataract – and it is important to determine whether this is a possible side-effect in humans before it occurs. Cataractogenesis is related to increases in intracellular free calcium and it is well known, for example, that muscarinic agonists cause both lens opacification and an increase in intracellular free-calcium levels[56]. However, what has not been clear is which muscarinic receptors are involved. Work with immortalized human lens epithelial cells and native lens cells demonstrate that both respond in a similar way to exogenous acetylcholine. However, whereas the response in the immortalized cells is M_3-receptor-mediated, with little or no contribution from any of the other subtypes of muscarinic receptor, in the native cells the response to acetylcholine is almost entirely mediated by M_1-receptors[57] (Fig. 5). These findings are supported by studies on gene expression, where native cells express almost exclusively M_1-receptor mRNA, whereas in immortalized cells only M_3-receptor mRNA can be detected[57].

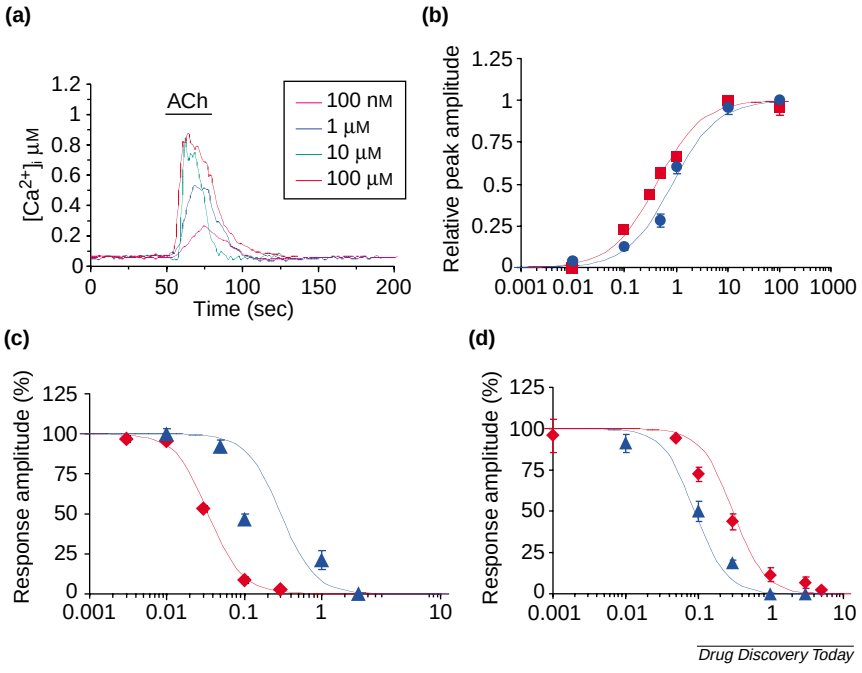

Figure 5. Effects of acetylcholine (ACh) on calcium mobilization in human lens epithelial cells and antagonism by muscarinic antagonists. **(a)** Effects of increasing concentrations of ACh on native lens epithelial cells (NLE cells). **(b)** Concentration-effect curves to ACh on NLE cells (■) and cultured HLE-B3 cells (●). **(c), (d)** Inhibitory effects of increasing concentrations of the muscarinic M_1 receptor-selective antagonist pirenzepine (◆) and the M_3 receptor selective antagonist AF-DX 384 (▲) on ACh-induced effects on NLE cells (c) and cultured HLE-B3 cells (d). Figure reproduced from Ref. 57 with the permission of the publisher.

Conclusions and future perspectives

To summarize, it is clear that human tissues have a wide range of applications in establishing ADME and safety profiles of development compounds. One perceived disadvantage of using fresh human tissues to study ADME is the inter-individual differences in expression of key enzymes and proteins involved in these processes. Although inter-individual variation can be problematic for selecting the right compounds from a battery of high-throughput screens, it can be 'controlled' by using pooled samples, for example, liver microsomes or cryopreserved hepatocytes, for metabolism screening. HTS is invaluable to the pharmaceutical industry for making in-house decisions on compound selection but, for data that might be incorporated in a submission to the regulatory authorities, an indication of inter-individual variability can be of value, bearing in

mind that the population ultimately receiving these drugs will exhibit such variability.

The use of native human tissue during later stages of drug discovery and development could usefully highlight the variation that might be expected in the clinic. This could be of paramount importance when investigating the potential for side-effects to new drugs, which often occur in particular individuals.

In the light of the pharmaceutical industry's persistently poor record of getting new drugs through the development phase to market, it appears that an increased awareness and use of human tissue profiling could provide at least some advance warning of troubles to come and increase the efficiency of drug discovery in the 21st century.

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