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Cells in focus

Hepatocytes: The powerhouse of biotransformation

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ABSTRACT

Liver is the most important organ involved in biotransformation of xenobiotics. Within the main organisational unit, the hepatocyte, is an assembly of enzymes commonly classified as phase I and phase II enzymes. The phase I enzymes principally cytochrome P450 catalyse both oxidative and reductive reactions of a bewildering number of xenobiotics. Many of the products of phase I enzymes become substrates for the phase II enzymes, which catalyse conjugation reactions making use of endogenous cofactors. As xenobiotic metabolising enzymes are responsible for the toxicity of many chemicals and drugs, testing the role of the biotransformation enzymes and the transporters within the hepatocyte is critical. New methodologies may be able to provide information to allow for better in vitro to in vivo extrapolation of data.

Cell facts

- Hepatocytes detoxify endogenous and exogenous substrates by the catalysis of phase I, principally cytochrome P450, and phase II biotransformation enzymes.
- These pathways can be altered by endogenous and exogenous factors.
- The ultimate disposition and behaviour of xenobiotics in the hepatocyte requires the interplay of membrane transporters for both intake and efflux.
- Testing the effect of drugs on hepatocytes and the role and impact of biotransformation pathways is a critical step in drug development and safety testing.

1. Introduction

Hepatocytes are the parenchymal cells of the liver, whilst they have many functions (Sell, 2003), importantly they are responsible for the biotransformation of both endogenous and exogenous lipid soluble compounds. Other functions of hepatocytes, whilst not the focus of this review includes nutrient homeostasis (glucose storage and synthesis, cholesterol uptake), filtration of particulates, protein synthesis (clotting factors, albumin), bioactivation (steroids) and formation of bile.

Prominent features of hepatocytes include a round nucleus and numerous mitochondria, suggesting the liver plays a significant role in energy metabolism. Hepatocytes also contain large amounts of endoplasmic reticulum (ER). The presence of the ribosome containing rough endoplasmic reticulum (RER) reflects a major function of hepatocytes – protein synthesis.

Together with the RER there is an extensive meshwork of smooth endoplasmic reticulum (SER), which incorporates large amounts of biotransformation enzymes, others are found in the cytosol. Hepatocytes are organised into plates separated by vascular channels or sinusoids. This structure is important in directing the excretion of the products of biotransformation away from the hepatocytes into bile and blood.

The biotransformation pathways are divided into phase I and phase II (Kitada et al., 1991). The cytochrome P450 (CYP) system is pivotal to the phase I system. The phase II enzymes are characterized by their ability to conjugate exogenous molecules using endogenous cofactors. Fig. 1 provides an overview of the biotransformation enzymes in the hepatocyte and the interaction of the biotransformation enzymes and the transporters.

2. Cell origin and plasticity

Hepatic organogenesis begins from the fourth week of gestation and by 12 weeks the SER is developing (Orme-Johnson and Ziegler, 1965). The pattern of enzyme development and the changes that occur in the enzymes present and their levels can have important implications in therapeutic efficacy of drugs and adverse events (Hines, 2008).

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Several hepatic functions such as biotransformation are regulated by a suite of nuclear receptors (PXR, CAR, PPAR and AHR), commonly called xenosensors as they bind endogenous and exogenous molecules (inducers) and thus initiate the phenomenon called induction (Lo Guidice et al., 1997). Additionally the activity of the biotransformation enzymes and the transporters can be inhibited.

Consequently biotransformation processes in hepatocytes are rather adaptive to changes in the internal *milieu*. The triggers for those changes can be exogenous drugs, other xenobiotics or endogenous steroid hormones and other endogenous molecules.

3. Functions

3.1. Protein synthesis

Within the RER critical proteins are synthesised, including albumin, fibrinogen and prothrombin as well as lipoproteins ceruloplasmin, transferrin and glycoproteins. Hepatocytes produce their own structural proteins and intracellular enzymes including the biotransformation enzymes. Other important roles of hepatocytes include carbohydrate and lipid metabolism and whilst they are not within the scope of this review, it is relevant to remind that the liver is the sole site of bile acid formation (Umeno et al., 1988).

3.2. Phase I enzymes

The cytochrome P450 (CYP) was identified after the publication of the key papers by Omura and Sato (1964a,b). This enzyme is characterized as a haeme-containing microsomal mono-oxygenase that is dependent on the availability of reducing equivalents from NADPH by the way of cytochrome P450 reductase which is also imbedded in the SER (Fig. 1). To date 107 genes encoding for CYPs in humans have been identified, with 18 families and 45 subfamilies. Of the 107 genes identified in humans, 57 have been isolated, identified and characterized (CPD, 2006).

CYPs are a major site for drug–drug interactions as they have a broad substrate specificity, which allows for competition between substrates for the same enzyme. Additionally inhibition (which may be mechanism based or direct) is dose related; at low concentrations the inhibitor may be relatively selective for a single CYP, and at high concentrations, the inhibitor may be relatively non-selective and several CYP enzymes may be inhibited. The inhibition of any CYP isozyme may be clinically significant but due to the nature of CYPs it is not always straightforward. For example, theophylline used in the treatment of asthma is metabolised by two pathways, N-demethylation which occurs via CYP1A2 and 8-hydroxylation which occurs via CYP1A2, CYP2E1 and CYP3A4 (Sarkar et al., 1992, 1994; Tjia et al., 1996). Inhibition of CYP1A2 and CYP3A4 by macrolide antibiotics may decrease the metabolism of theophylline, increasing serum concentrations causing toxicity.

Induction of drug metabolism may arise as a consequence of increased synthesis, decreased degradation, activation of pre-existing components or a combination of these processes. Induction of CYPs is said to occur when there is an increase in the amount and activity of the enzyme. The time for induction is not only dependent on the half-life of the inducing agent but also the time course for enzyme degradation and new enzyme production.

CYPs are highly polymorphic, i.e. there are several upstream, downstream, exon and intron SNPs, insertions, deletions and partial or total gene deletions. For example, there are close to 100 gene variants of CYP2D6. Because many of these changes result in functional consequences, i.e. lack, decrease, increase of activity, a huge variability of CYP2D6-associated activities is detected in any population. The same is true, although perhaps not to the same extent as with CYP2D6, with most xenobiotic-metabolising enzymes including phase II enzymes.

Flavin monooxygenases (FMO), like CYP require NADPH and O_2, many of the reactions catalysed by FMO also can be catalysed by CYP. FMO oxidize the nucelophilic nitrogen, sulphur and phosphorus atoms of xenobiotics. Of the five forms of FMO, FMO3, FMO4 and FMO5 are expressed in high levels in human liver.

Epoxide hydrolase (EH), found mainly in the cytosol of hepatocytes catalyses the reaction of converting an epoxide (often produced within the hepatocyte by CYP) into a dihydrodiol. This group of phase I enzymes, contains five distinct forms in mammals. These enzymes may play an important role in detoxifying electrophilic exposides that may bind to proteins and nucleic acids and cause cellular toxicity and genetic mutations.

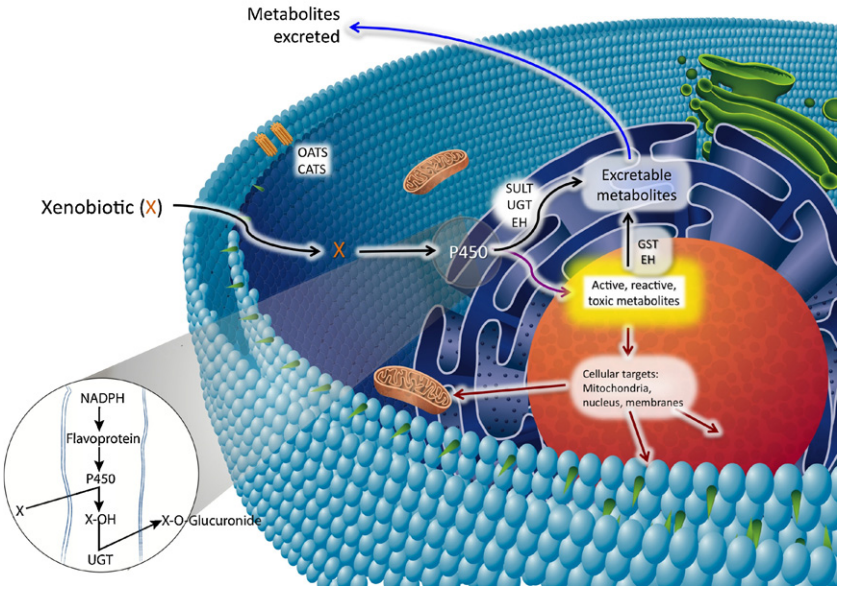

Fig. 1. Processing of xenobiotics in the hepatocyte. Cytochrome P450 (CYP) and other hepatic enzymes such as epoxide hydrolase (EH), conjugating enzymes, UDP glucuronyl transferase (UGT), sulphotransferase (SULT) or glutathione S-transferase (GST) are important in metabolic handling of xenobiotics. The enzymes depend on molecular oxygen and NADPH (CYP), UDPG (UGT), PAPS (SULT) and reduced glutathione (GSH) (GST). The cellular membrane has many transporters, OATS, ABC transporters and OCTS, which are important for the transmembrane flux of many xenobiotics including the products of the conjugating enzymes.

3.3. Phase II enzymes

Important phase II enzymes include but are not limited to those highlighted here.

Glucuronosyl transferase or uridine diphospho glucuronyl transferase (UGT), of which there are 16 isozymes in humans, catalyses the conjugation of a wide variety of compounds which are often products of CYP catalysed reactions. UGT is strategically placed in the SER in close proximity to CYPs (Fig. 1). Therefore many products of CYP catalysed reactions may become substrates of UGT before they have an opportunity to diffuse out from the lipoidal milieu of the endoplasmic reticulum.

Sulphotransferase (SULT) catalyses the transfer of a sulphonate group from a high-energy donor molecule 3′-phosphoadenosine-5′-phosphosulphate (PAPS) to a substrate containing an alcohol or an amine group. There are at least 11 distinct enzymes that belong to this group. Many drugs, steroids and xenobiotics gain an alcohol or amine group as a result of CYP catalysed reactions and become suitable targets of sulfonation by SULT.

N-acetylation catalyses the conversion of xenobiotics containing an aromatic amine or a hydrazine group to aromatic amides or hydrazides, respectively. Many N-acetylated metabolites are less water soluble than the parent compound. N-acetylatranferase (NAT) in humans consists of two closely related proteins (NAT1 and NAT2) that are encoded for by highly polymorphic genes.

Glutathione S-transferase (GST) is a family of enzymes that represent quantitatively a very significant proportion of hepatic protein that can also function as transport proteins. GST is considered largely, cytosolic however, important isozymes are found also in microsomes, mitochondria and nuclei (Klaassen, 2008). Glutathione conjugation is an important biotransformation reaction for many compounds, including halogenated aromatics, lipid peroxides and arene oxides (epoxides) produced by CYP catalysed reactions.

3.4. Transporters

A large number of transporters affect the movement of xenobiotics over the cell membrane, thus affecting their intracellular concentrations and disposition. The estimation of the role of transporters is rather difficult, because xenobiotics move across cell membrane also by passive diffusion according to their physicochemical properties, as well as by active transport.

Important transporters include the anion transporters OATP1B1/SLCO1B1 and OATP1B3/SLCO1B3, organic cation transporter and the ATP-binding cassette (ABC) transporters which includes P-glycoprotein, MRP2 and BCRP.

As with the biotransformation enzymes, genetic polymorphisms occur with transporters. Additionally activity levels of the transporters can be altered by drug–drug interactions causing inhibition or induction.

4. Associated pathologies

Pathological considerations are relevant to biotransformation in two ways: liver disease impacts on the biotransformation enzymes and secondly the enzymes can both protect the liver or facilitate liver toxicity of xenobiotics.

The most common and highly dose dependent liver injury facilitated by biotransformation enzymes is exemplified by paracetamol (Fig. 2). Increasing the dose of paracetamol can overwhelm both the SULT and UGT catalysed pathways. When this happens the surplus paracetamol is metabolised by CYP into highly reactive NAPQI, which is detoxified by GST. Sufficiently high doses of paracetamol can deplete GST cofactor GSH and NAPQI reacts increasingly with cellular macromolecules resulting in extensive damage to the hepatocyte.

4.1. Hepatocytes in drug development

Nearly 50% of all drugs fail in the post-marketing phase due to unexpected toxicity or alteration or metabolism issues due to interaction with genetic factors, environmental factors, nutrition, patient condition or other drugs (Abboud and Kaplowitz, 2007; Lee, 2003). Investigating the role of the biotransfomation enzymes is critical to identify the generation of toxic metabolites, and determine the pharmacokinetic properties of xenobiotics. For this reason hepatocytes are important tools in drug development and testing.

Viable hepatocytes for toxicity testing are either used as tissue slices or as isolated cell populations, in both systems hepatocytes exhibit rapid phenotypic changes and limited survival. Whilst human hepatocytes in culture show active levels of major biotransformation enzymes (Donato et al., 1999; Rodríguez-Antona et al., 2002), differences amongst preparations are found due to different donors (LeCluyse, 2001; Donato et al., 1995). Whilst hepatocytes account for the vast majority of the liver volume (about 80%), other cells such as Kupffer cells may be necessary for cofactor supply.

The expression of biotransformation enzymes declines over time, with an initial rapid loss in mRNA (20–40%) at 4–6 h after culturing, which precedes the decrease in CYP protein and activity (Rodríguez-Antona et al., 2002; Gómez-Lechón et al., 2004). Importantly not all enzymes degrade at the same rate due to stability of the individual isozymes (Gomez-Lechon et al., 2007). For some CYPs this loss becomes evident after a few days (CYP2E1 and CYP3A4), whilst others remain unaffected (CYP1A2 and CYP2C9) (George et al., 1997). These limitations can be quantified but not overcome by characterizing the cells.

4.2. New methodologies

Embryonic and non-embryonic stem cells have been investigated as potential sources of mature hepatocytes, though the limited phenotype expression prevents the application of these cells in routine testing. To date only a few studies have analysed the biotransformation functions, mostly CYPs with low levels of mRNA and/or activity being detected for several enzymes (Guguen-Guillouzo et al., 2010).

Metabolically competent permanent cell lines such as HepaRG cells are currently under intensive study, as they may provide a continuous, proliferating hepatocyte-mimicking cell line not dependent on liver donors. This cell line, originally derived from a human hepatocellular carcinoma, has the ability to differentiate into both the biliary and hepatocyte lineage (Beaune et al., 1987; Cerec et al., 2007).

HepaRG cells are known to express biotransformation enzymes, with CYPs, phase II enzymes and both influx and efflux transporters for up to four weeks (Aninat et al., 2006). Induction of several CYP enzymes has also been reported and the intrinsic clearance rates are comparable with primary human hepatocytes (Lübberstedt et al., 2011). Whilst this cell line may be a useful tool that can be employed in toxicity testing, there are limitations. The HepaRG cell line has been derived from a single donor and therefore it does not represent the high level of inter-individual variability that is known to exist with the biotransformation enzymes. Additionally the 3-dimensional arrangement of the liver is of key importance in understanding how the biotransformation enzymes interact and this is a 2-dimensional system.

Transgenic mice with human CYPs have been developed and can be used for CYP investigations, though these mice usually express

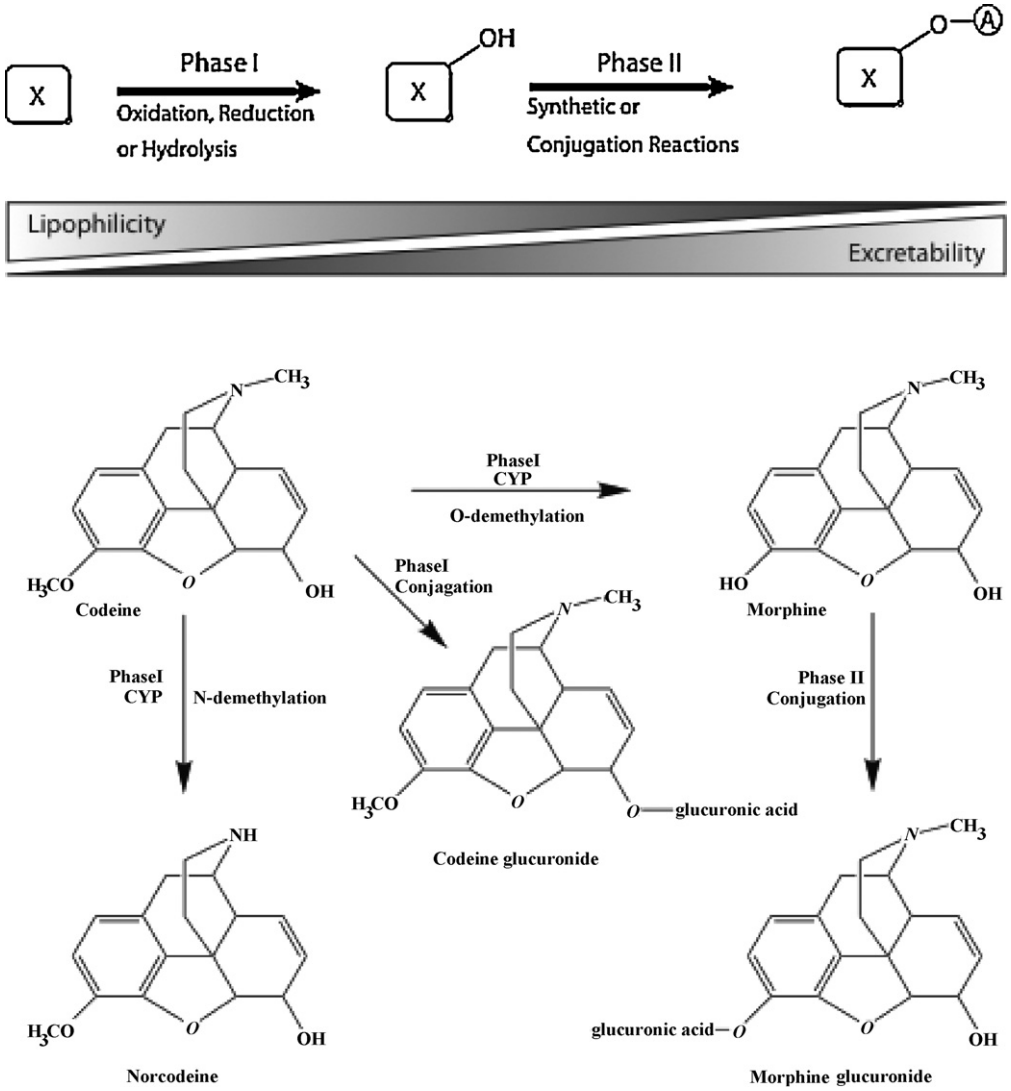

Fig. 2. The biotransformation of a large number of small organic xenobiotics can be represented in simple terms of phase I (oxidation, reduction, hydrolytic) reactions which are often followed by conjugation or synthetic reactions termed phase II reactions by R.T. Williams (2). Codeine is a good example illustrating phase I oxidation followed by conjugation with glucuronic acid. As codeine itself contains a suitable functional group it can also bypass phase I reactions and be conjugated directly.

one humanized isoenzyme, for example CYP2D6 or CYP3A4 (Zhang et al., 2003; Robertson et al., 2003). Chimeric mice with humanized livers can be used to predict the human in vivo interaction of a chemical with the CYPs and the metabolites generated. The first reports of substantial repopulation of a mouse liver with human hepatocytes were published in 2001 (Dandri et al., 2001; Mercer et al., 2001). Investigations with these chimeric models have identified expression of human-specific mRNA for CYP1A1, CYP1A2, CYP2C9, CYP2D6 and CYP3A4 (Katoh et al., 2004).

Chimeric mice are also of interest as they express phase II enzymes and the transporters (Kamimura et al., 2010; Strom et al., 2010). This would allow for a more detailed metabolic profile and therefore more realistic extrapolation to human in vivo studies.

Recent developments in microfabrication technologies coupled with cell culture have made it possible to develop "cells on a chip", which have been used to mimic biological systems. Multiple-chamber microscale cell culture systems have been applied also to hepatocytes together with other cell types in the liver.

Microfluidics allow for the continuous supply of nutrients and waste removal which enables the environment to be more stable (Wu et al., 2010). A microfluidic 3-dimensional system that is capable of characterizing drug metabolites and drug toxicity simultaneously has been reported (Ma et al., 2009). Importantly these systems also maintain the phenotype making them more promising for toxicity testing.

Before these systems can become routine several key questions need to be answered. The perfusion cell culture system needs to be investigated so that the removal of waste does not interrupt cell to cell communication and the delivery of drugs needs to be uniform. The technology needs to be further developed to allow these systems to become more high throughput but these systems still hold great potential and may be used in conjunction with more rapid pre-clinical testing.

Calling hepatocytes 'the powerhouse of biotransformation' is appropriate considering the ability of these cells to metabolise a vast number of xenobiotics including drugs, food constituents and additives, food and environmental contaminants and many other chemicals including those that may be synthesised in future. Investigating the role that hepatocytes play in biotransformation and overall pharmacokinetics and dynamics will improve not only our understanding of systems biology but also lead to potentially safer drugs.

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