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Revisión

In vitro investigation of drug metabolism and toxicity in man

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ABSTRACT

The pharmaceutical industry is committed to marketing safer drugs with fewer side effects, predictable pharmacokinetic properties and quantifiable drug-drug interactions. Drug metabolism is a major determinant of drug clearance and interindividual pharmacokinetic differences, and an indirect determinant of the clinical efficacy and toxicity of drugs. From a commercial perspective, it is desirable that poorly behaved compounds are removed early in the discovery phase rather than during the more costly drug development phases. As a consequence, over the past decade, *in vitro*-based strategies in lead optimization screening in conjunction with ADMET screening studies have been incorporated earlier in the drug discovery

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Abbreviations:
ADMET: Absorption-Distribution-Metabolism-Excretion-Toxicity.
EMEA: European Agency for the Evaluation of Medicinal products.
FDA: Food and Drug Administration.
P450: Cytochrome P450 system.

phase. At present, the use of human *in vitro* hepatic models at early preclinical stages means that the process of selecting drug candidates is becoming much more rational. Several *in vitro* tools are available to address key issues at the earliest stages of drug development for a better candidate selection and hepatotoxicity risk assessment.

Key words: Cytochrome P450.—Drug metabolism.—Drug-drug interaction.—Hepatotoxicity.—Human hepatocytes.—Microsomes.

RESUMEN

Investigación *in vitro* del metabolismo y la toxicidad de fármacos en el hombre

El interés de la industria farmacéutica es incrementar la seguridad de los nuevos fármacos, disminuir sus efectos secundarios, conocer sus propiedades farmacocinéticas e identificar las posibles interacciones fármaco-fármaco. El metabolismo de los fármacos es un factor determinante en su aclaramiento, es responsable de la variabilidad interindividual de la farmacocinética y en consecuencia, de la variación en el efecto farmacológico y la toxicidad. Desde la óptica comercial, lo deseable es que los fármacos con propiedades no adecuadas sean descartados en las fases más tempranas del desarrollo, y no en las fases posteriores con mayor coste económico. Como consecuencia de esto, en las últimas décadas se han incorporado estrategias basadas en modelos *in vitro* en combinación con estudios de ADMET, para la selección de nuevos fármacos cabeza de serie durante fases muy tempranas del desarrollo. Actualmente, la utilización de modelos hepáticos humanos *in vitro* en las fases preclínicas supone un proceso de selección de nuevas moléculas candidatas a fármacos mucho más racional. Existen varios modelos *in vitro* para abordar estas cuestiones en las etapas tempranas del desarrollo de fármacos para optimizar la selección de moléculas candidatas y la evaluación del riesgo de hepatotoxicidad.

Palabras clave: Citocromo P450.—Metabolismo de fármacos.—Interacción fármaco-fárrmaco.—Hepatotoxicidad.—Hepatocitos humanos.—Microsomas.

INTRODUCTION

Drugs and other xenobiotics usually have a low solubility in aqueous systems and require biotransformation to metabolites that are more hydrophilic and more readily eliminated. Typically, liver drug metabolism occurs in two phases: Phase I and Phase II. Phase I of biotransformation is the oxidative pathway in which the

compound undergoes oxidation to a more polar substance. Cytochrome P-450 (P450)-depending monooxygenases and flavin monooxygenases, are major role players in the oxidative metabolism of xenobiotics and endogenous compounds. This process is followed by the Phase II reactions in which metabolites are further conjugated by hepatocytes with endogenous molecules by glucoronidation, sulfation, methylation, acetylation and mercapture formation, rendering derivatives that are much more soluble, thus facilitating their elimination (1-3). Biotransformation reactions generally follow a detoxification process rendering metabolites inactive. Nevertheless, many drug intermediary products generated during metabolism are highly reactive and toxic, causing hepatotoxicity (4-6). Metabolism is the major determinant of drug clearance and interindividual pharmacokinetic differences, and the indirectly determinant of the clinical efficacy and toxicity of drugs. Many potential drug candidates are rejected in drug discovery given the undesirable pharmacokinetics that can result in an inadequate concentration of the drug at the site of action and/or great variations in clinical response and adverse effects.

Drug regulatory agencies, such as the Food and Drug Administration (FDA) and the European Agency for the Evaluation of Medicinal Products (EMEA) (7), have issued different guidelines to enhance the importance of ADME studies in the drug development process. The pharmaceutical industry is required to market safer drugs with fewer side effects, predictable pharmacokinetic properties and quantifiable drug-drug interactions. Therefore, companies are increasingly interested in optimizing these properties during early drug development phases (Figure 1). Rapid biotransformation, resulting in a short exposure to the pharmacologically active parent compound and the formation of active or toxic metabolites, is not usually welcomed. Alternatively, an extremely stable drug may pose a potential problem of drug-drug interactions and toxicity. Medicinal chemists are primarily concerned in designing molecules that will not only offer the desired activity, but suitable potency and duration of action which are influenced by pharmacokinetic properties. Consequently over the past decade, *in vitro*-based strategies in lead optimization screening in conjunction with ADMET screening studies have been incorporated earlier in the drug discovery phase (8, 9).

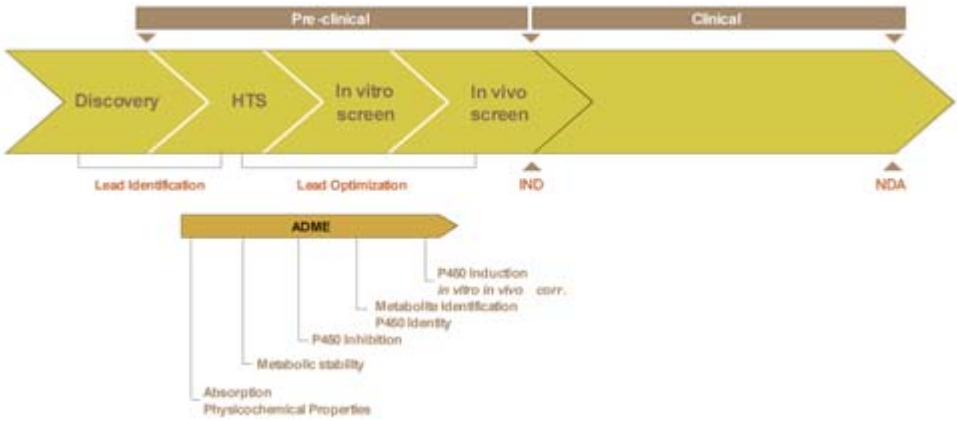

FIGURE 1. ***Drug development process depicting the different types of ADME studies that could be performed at the various stages.*** *Investigational New Drug (IND). New Drug Application (NDA).*

KEY ISSUES TO BE ADDRESSED AT EARLY STAGES OF DRUG DEVELOPMENT

After intake, drugs are absorbed and distributed among the tissues and body fluids and then eliminated or cleared, mainly by the liver and kidneys. Therefore, the development of a new drug requires an exhaustive characterization of not only its pharmacological activity, but also knowledge of major enzymes involved in the metabolite formation, and the potential enzyme-inhibiting or enzyme-inducing properties of the drug. Therefore, over the last decade, *in vitro*-based strategies in lead optimization screening combined with ADMET screening studies have consequently been incorporated earlier in the drug discovery phase (10).

Several metabolic key issues should be addressed at very early stages of drug development for a better selection of the safest and most effective drug candidates (Figure 2):

a) Drug metabolic stability and metabolic profile.

b) Metabolite identification and structure clarification.

c) Prediction of *in vivo* pharmacokinetic parameters from *in vitro* data.

d) Identification of the P450 enzymes involved in drug metabolism.

e) Interspecies comparison of the metabolic profile of a drug to select the animal. species closest to man for preclinical studies.

f) Drug-drug interactions due to enzyme induction/inhibition.

g) Drug toxicity associated with drug metabolism.

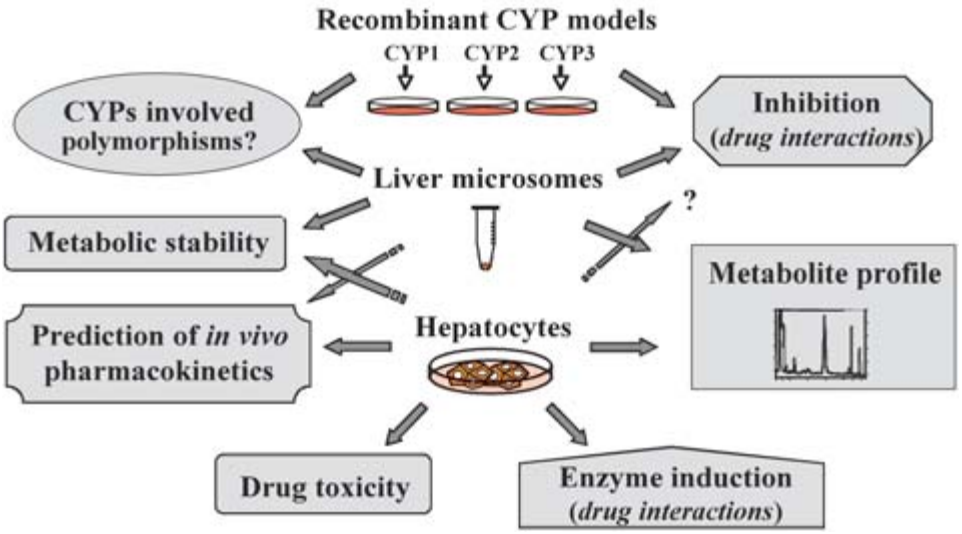

FIGURE 2. ***Major applications of in vitro hepatic models during the development of new drugs.***

DRUG METABOLIC STABILITY AND METABOLIC PROFILE AND METABOLITE IDENTIFICATION

Using *in vitro* methods enables us to determine and predict the metabolic stability of NCEs, which can be an important contributor

for a good pharmacokinetic profile as well as a risk for drug-drug interactions. Metabolic stability is defined as the susceptibility of a chemical compound to biotransformation, and is expressed as *in vitro* half-life (t(1/2)) and intrinsic clearance (CL(int)). Based on these values, *in vivo* pharmacokinetic parameters, such as bioavailability and *in vivo* half-life, can be calculated when other data concerning the volume of distribution and fraction absorbed are available (11). Metabolic stability assays can be easily investigated by incubating new chemicals with fully competent metabolic models and performing sensitive chromatographic analysis (e.g. HPLC-MS/MS) of the incubation mixtures (12). In the early phases of chemical screening, metabolic rates are estimated by measurement of the disappearance of the test compound as metabolites are usually unknown. At advanced stages, formation of metabolites is also analyzed. Human liver microsomes, hepatocytes, and cDNA-expressed P450 enzymes are commonly used (Figure 2). A major disadvantage of using recombinant models expressing a single enzyme for the study of metabolic stability of a drug is the lack of other phase I and phase II enzymes. Human liver microsomes contain high levels of P450s and other drug-metabolizing enzymes (flavin monooxygenase, UDP-glucuronyltransferases, epoxide hydrolase). The metabolites identified after a short incubation with microsomes coincide with those reported as the major metabolites in human *in vivo* studies (13). The major limitation of microsomes is that they lack phase II cytosolic enzymes (glutathione S-transferases, sulfotransferases, soluble epoxide hydrolases, alcohol dehydrogenase, xanthine oxidase, etc.). Human hepatocytes represent a more complete system with physiological levels of cofactors, natural orientation for linked enzymes and intact membranes to allow for the modeling of intracellular drug concentrations (14, 15) (Figure 2). Moreover, the restricted accessibility of suitable human liver samples has greatly hindered the widespread use of human hepatocytes for drug metabolism studies. Recently, this scarcity has been countered by the increasing availability of metabolically competent cryopreserved human hepatocytes and by the use of optimized metabolic assays in hepatocytes cultured in multiwell plate formats (16, 17). Later in non clinical development, the ADME flow chart focuses on metabolite profiling in different species and metabolite identification. Significant metabolic interspecies

differences between animals and man exist, not only because of the different P450 enzymes expressed in man and other species, but also because of their relative abundance (18-20). Therefore, metabolite profiles in different animal models (i.e. microsomes or hepatocytes from several species) are analyzed and compared with the human profile toward a more astute selection of animal species for subsequent pharmacokinetic or toxicological studies. As metabolites may be inert, pharmacologically active or reactive, drug metabolite profile evaluation and metabolite identification are essential to design new safer drug candidates with improved ADME capabilities. Incubating the drug candidate with recombinant P450s individually expressed in various host systems can generate large amounts of metabolites for chemical structure identification (Figure 2). Analytically speaking, liquid chromatography tandem mass spectrometry is the most widely used tool to identify metabolites and determine both structure and metabolite profiles (21).

PREDICTION OF *IN VIVO* PHARMACOKINETIC PARAMETERS FROM *IN VITRO* DATA

Human liver-derived models are invaluable tools in elucidating the pharmacokinetic parameters of a drug candidate and the selection of lead compounds with favorable properties during the drug discovery and development process (22, 23). Clearance of a drug from the body depends on the intrinsic ability of the organs, such as liver and kidney, to metabolize and excrete. Systemic clearance of a drug that is eliminated by hepatic metabolism is a function of the hepatic blood flow and the intrinsic clearance of the liver (CLint, *in vivo*), which is defined as the ability of the liver to remove xenobiotics from the blood in the absence of other confounding factors. Disease conditions of the liver or administration of drugs that are inducers or inhibitors of P450 can therefore influence systemic clearance. CLint, *in vitro* is a measure of enzyme activity towards a drug which is not influenced by other physiological determinants of liver clearance, such as hepatic blood flow or drug binding to blood proteins (24). As with all clearance terms, it has units of volume rate and acts as a proportionality constant to describe the relationship between the metabolism rate of a drug and

its concentration at the enzyme site. Two strategies underlying the prediction of *in vivo* hepatic drug metabolism from *in vitro* data have been defined (24, 25): the metabolite formation method, the most widely used, and the more recently adopted substrate depletion approach, where the intake of the parent drug is monitored over time (25). From a biochemical point of view CLint, *in vitro* can be considered in terms of the enzyme parameters of the Michaelis-Menten equation (24, 26). Enzyme kinetics data must be obtained under linear conditions with regard to the enzyme concentration and incubation time, that is, the period when V_0 is maintained. Once CLint, *in vitro* is obtained from Michaelis-Menten constants (Km and Vmax), reasonable estimates of *in vivo* hepatic clearance (CLint, *in vivo*) can be obtained by using appropriate scaling factors and modeling (24, 26, 27). Mathematical physiological models (well-stirred, parallel tube, distributed and dispersion models) of hepatic drug clearances, which use both anatomical and physiological data, have been appraised in relation to their utility in predicting drug removal by the liver (28-30).

IDENTIFICATION OF P450 ENZYMES INVOLVED IN DRUG METABOLISM

Early identification of P450s responsible for the metabolism of new molecules is important in drug discovery in order to minimize the role of polymorphic enzymes leading to inter-individual variation and potential drug-drug interactions. Pooled human liver microsomes are the most frequently model used for this purpose (31, 32). A major advantage of these *in vitro* systems is that P450 enzymes are present in their physiological relative proportions and can interact with other essential proteins required for P450-catalyzed oxidations (i.e. NADPH cytochrome P450 reductase, cytochrome b5). By the use of selective chemical inhibitors for individual P450s, the major metabolic pathways for a new drug can be either readily demonstrated or ruled out. The incubations in which the metabolism of the test drug is reduced suggest the involvement of the P450 enzyme, whose activity is affected by the inhibitor. One drawback that these assays present, even when using potent inhibitors, is that it is not possible to completely and selectively inhibit a single P450 activity.

In recent years, recombinant human P450 systems have been increasingly used for this purpose (Figure 2). Major limitations inherent to recombinant models are that concentrations of P450 enzymes are far in excess of their relative amount in the human liver, and that the secondary metabolism cannot be identified. However, such disadvantages have not appeared to hinder the utility of cDNA expressed enzymes to P450 reaction phenotyping. Incubating the compound with each separate recombinant P450 provides information about the role of individual P450s in metabolite formation (32, 33-37). However, the degree of involvement of a P450 in a particular reaction *in vivo* can neither be estimated nor can the metabolic profile of a drug in man be anticipated. Different strategies based on the combined use of *in vitro* models showing a full contingent of P450 enzymes (i.e. human liver microsomes, primary human hepatocytes) and P450 recombinant models have been proposed to mathematically reconstruct the relative contribution of each P450 enzyme in the metabolism of a given compound (35-39).

DRUG-DRUG INTERACTIONS DUE TO ENZYME INDUCTION/INHIBITION

Pharmacokinetic interactions occur when the disposition (i.e. absorption, distribution, metabolism and excretion) of a drug is altered by another (40). One of the most important pharmacokinetic factors that control drug action is the rate of metabolic transformation. Hence, interactions that result in changes in the rate of drug metabolism can be of great clinical significance. Many drugs can inhibit, induce and alter relative amounts of different P450 enzymes. A strong inhibition/induction of P450 activities by a molecule is expected to seriously interfere with the metabolism of other drugs administered simultaneously or subsequently. These changes in *in vivo* metabolism rates can lead drug concentrations to fall outside their therapeutic window, implication a serious risk of drug-drug interactions. The high cost associated with drug development programs has focused attention on predicting, identifying and avoiding inhibitory potential early in the discovery process. *In vitro* studies, which are generally inexpensive and readily carried out, must serve as preliminary screenings to rule out the need

of *in vivo* testing. The objective of *in vitro* screening of P450 inhibition properties of drug candidates is to exclude potent inhibitors from further development. P450 enzymes play a crucial role in the metabolism of drugs and, therefore, P450 inhibition is the most important mechanism for metabolic drug-drug interactions (41, 42).

Microsomes and cDNA-expressed enzymes are the preferred test systems as they are more readily available than human hepatocytes (Figure 2). Assays are based on the analysis of potential reductions in the metabolism of an appropriate P450 probe substrate in the presence of various concentrations of the tested compound (11, 13). For the purpose of high throughput P450 inhibition screening, a variety of strategies based on fluorescence, LC-MS and radiometry approaches have been developed (43-45). Since several enzymes may be involved in the metabolism of a compound, the use of recombinant models expressing a single P450 may lead to an overestimation of the inhibitory effect of a given drug (46). A major limitation in making conclusive statements from assays in microsomes or recombinant enzymes is that ultimately *in vivo* metabolism is complicated by the role of processes missing in subcellular models. Drug transport across membranes, further metabolism by cytosolic enzymes, or binding to intracellular proteins can be determinant in the actual concentration of the substrate and inhibitor available to the enzyme (41, 47). Assays performed in intact cells could be more predictive (47).

Drug-drug interactions can also occur as a consequence of P450 induction. Metabolic interactions due to enzyme induction are far less frequent than those caused by inhibition; however, their consequences can be clinically relevant. A drug with inductive properties can accelerate its own metabolism or those of other co-administered drugs, resulting in either therapeutic inefficacy or in an exaggerated response. Screening of inducers cannot be done in microsomes or recombinant models as it requires a cellular system that is fully capable of expressing genes (Figure 2). Currently, primary hepatocytes are still the unique *in vitro* model for global examination of the inductive potential of drugs (14, 48, 49). After 24-72 h incubation with the test compound, P450 induction is monitored as its increases in enzyme activity (using specific substrates) or mRNA levels (by quantitative RT-PCR). Results are

compared to those of untreated cells (Figure 3). Different concentrations of the drug, covering a large range of concentrations, are recommended to assess the inductive potential of a new chemical.

Generally, if a negative results from *in vitro* inhibition/induction assays is obtained (no identified interaction), the study of potential clinical interactions is not needed. *In vitro* experiments should be conducted at similar concentrations to the relevant *in vivo* concentration for the optimization of the clinical assays.

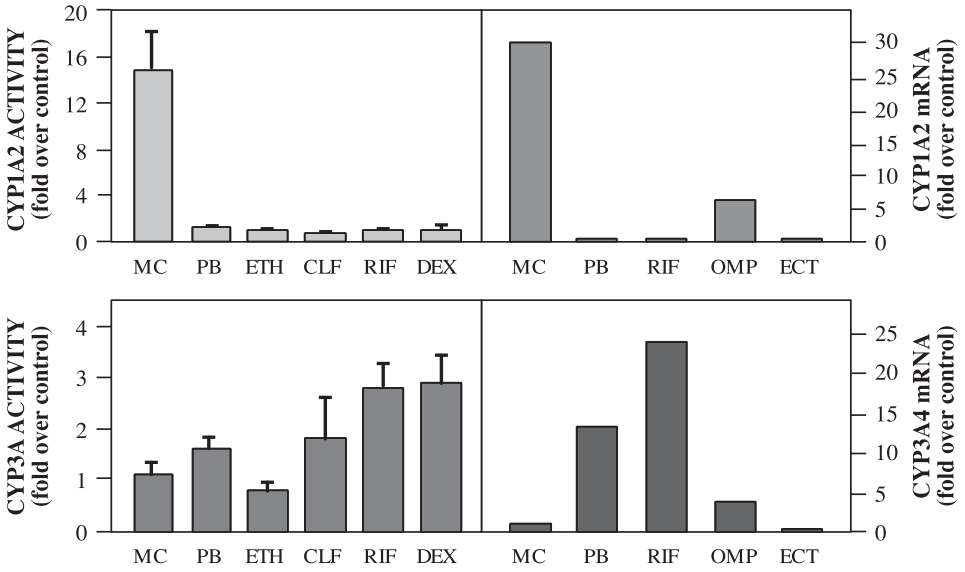

FIGURE 3. ***Effects of model inducers on P450 enzymes in cultured human hepatocytes.*** *After 24 h in culture, human hepatocytes were exposed to 2 μM 3-methylcholanthrene (MC), 1 mM phenobarbital (PB), 100 mM ethanol (ETH), 1 mM clofibric acid (CLF), 50 μM rifampicin (RIF), 1 μM dexamethasone (DEX), 50 μM omeprazole (OMP), or 1 nM ecteinascidin (ECT), and P450 activities and mRNA levels were measured 48 h later. Activities were determined in three different hepatocyte preparations by the use of selective substrates: 7-methoxyresorufin (CYP1A2) and testosterone (CYP3A4). Specific CYP mRNA levels were quantified by quantitative RT-PCR in two different cultures. Results are expressed as fold increases over corresponding activity or mRNA values in controls (untreated cells).*

DRUG-INDUCED HEPATIC INJURY

Substances capable of producing liver damage and, more specifically, hepatocyte damage are known as hepatotoxins. *Intrinsic hepatotoxins* are substances that exert their effects in all individuals, in a dose-dependent and hence predictable manner. These toxins can interfere directly with cell metabolism (*active hepatotoxins*) (50) or become toxic once they have been biotransformed (*latent hepatotoxins*). *Idiosyncratic hepatotoxicity*, on the other hand, may be the consequence of an abnormal metabolism of the drug by susceptible individuals (*metabolic idiosincrasy*) or be elicited by an immune-mediated hepatocyte injury (*allergic hepatitis*). The former has a geno- or phenotypic basis that results in the over/under expression of drug metabolizing enzymes, a different drug metabolism pattern and eventually the abnormal production of a toxic metabolite. This type of idiosyncratic toxicity is dose-dependent in susceptible individuals. Idiosyncratic drug toxicity is a rare human-specific event and therefore not detectable in experimental animals and impossible to be studied in clinical trials (51). Some xenobiotics are electrophilic in nature, and others are bioactivated by the liver to highly reactive metabolites generally more toxic than the parent compound, which is the key to many toxic phenomena (50, 52, 53). To minimize these effects, hepatocytes have effective defence mechanisms, and ultimately it is the balance between bioactivation, detoxification and defence/repair mechanisms that determines whether a compound will or will not elicit a toxic effect.

Figure 4 summarizes the molecular events that can be involved in hepatocyte toxicity: 1) The impairment of the biochemical functions of hepatocytes by the drug or by any of its stable metabolites is the first possible mechanism of hepatotoxicity (52-54). 2) The mitochondrion is a frequent target of hepatotoxic drugs and the alteration of its function has immediate effects on the energetic balance of cells (54). Depletion of ATP is, in fact, an early event in the course of drug-induced toxicity that precedes the irreversible stages of cell injury (56). 3) Lipid peroxidation. It is a free radical process leading to the oxidative degradation of lipids that finally may disrupt the structure and functionality of the cell membranes (56). 4) Alteration of intracellular Ca^{2+} concentration. Intracellular

calcium participates in many cellular functions and its levels should be perfectly regulated to obtain a proper cell function. Many substances can interfere in intracellular calcium homeostasis control, thus leading to cell malfunction and death (50, 57). 5) Oxidative stress is produced by compounds able to undergo repeated oxidation and reduction cycles within the cell (58). This redox cycling causes the continuous production of reactive oxygen species (e.g. superoxide anion) and depletion of GSH and nicotinamide nucleotide pools, with a concomitant increase in lipid peroxidation and intracellular Ca^{2+} accumulation. Finally, biotransformation of xenobiotics can also result in the formation of intermediates capable of covalently binding to cell macromolecules (proteins, DNA and RNA) to form stable drug adducts (58).

Cytotoxicity end-points (cell viability assays: MTT, neutral red uptake tests, etc; cell membrane permeability alteration: enzyme leakage; etc.) represent a first approach to assess hepatotoxicity, but evaluation of these parameters alone may leave out of consideration xenobiotics that impair cell function without causing cell death. This may not be critical for the hepatocyte itself, but can be of toxicological significance for the whole organism (59). By examining the effects on hepatocyte-specific metabolism, it is possible to find out whether relevant hepatic specific functions become altered by the presence of a xenobiotic. Currently, several metabolic parameters, representative of the liver's most characteristic functions, should be evaluated, namely, gluconeogenesis, glycogen metabolism, ureogenesis, plasma protein synthesis, synthesis of VLDL, etc. In general, metabolic parameters are more sensitive to the toxic effect of hepatotoxins than cytotoxicity indicators (60). Concentrations to which cells are exposed for cell metabolism studies should not cause perceptible cell death (they should be up to the MNTC).

The ultimate goal of *in vitro* experiments is to generate the type of scientific information needed to identify compounds that are potentially toxic to man for which purpose not only the design of experiments but also the interpretation of results are essential. Even simple parameters for assessing cell toxicity have yielded promising results when comparing *in vitro* effects with human toxicity as false negatives are infrequent in compounds that are toxic without biotransformation.

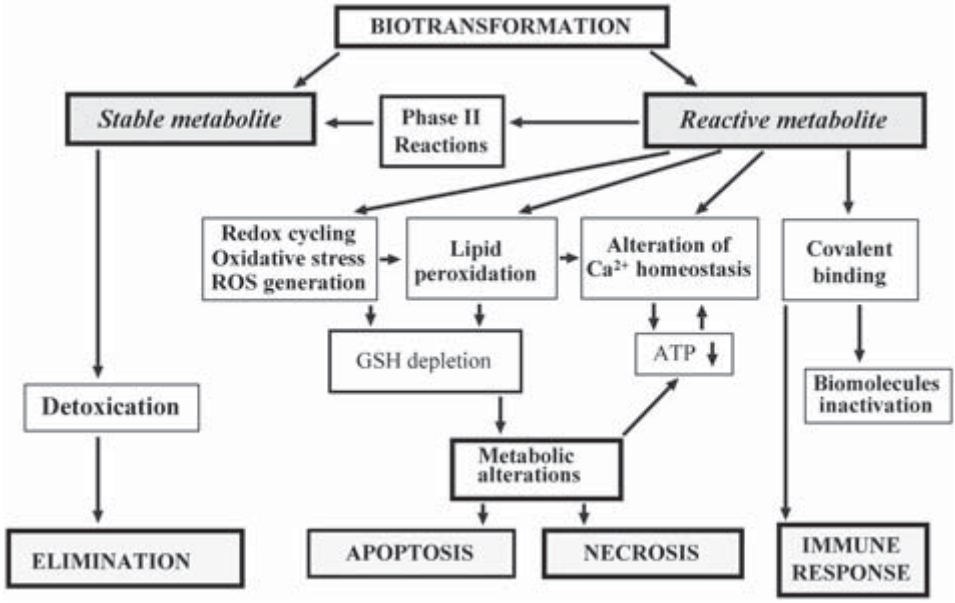

FIGURE 4. ***Mechanisms of drug hepatotoxicity.*** *Different mechanisms can be involved in hepatocyte toxicity: Mitochondrion is a frequent target of hepatotoxic drugs and the alteration of its function has immediate effects on the energetic balance of cells (depletion of ATP). Lipid peroxidation, oxidative stress, alteration of Ca^{2+} homeostasis and covalent binding to cell macromolecules are the molecular mechanisms more frequently involved in the toxicity of xenobiotics.*

The monitoring of gene expression of cells exposed to toxic xenobiotics (toxicogenomics), has gained great popularity among researchers (61-63). The ease with which thousands of genes can be measured has led most of the scientists to register changes of as many genes as possible with the hope that an in-depth biostatistical analysis would reveal which of that gene were clearly linked to toxic events. It is hoped that through a better understanding of cellular mechanisms of toxicity, combined with mechanistically directed toxicogenomic analysis, the accuracy and predictivity of *in vitro* screening for toxicants could be greatly improved.

VARIABILITY OF DRUG METABOLIC CAPABILITY IN HUMANS

Variability of drug metabolism rates is a consistent observation in human populations. Considerable differences can be observed among «extreme» individuals. Progressive advances in the knowledge of metabolic routes and enzymes responsible for drug biotransformation have contributed to understanding the great metabolic variations existing in human beings. Phenotypic and genotypic differences in the expression of the enzymes involved in drug metabolism are the main causes of this variability. It is well documented that P450 enzymes are polymorphically expressed (59, 64, 65). Mutations of P450 genes result in allelic variants causing defective, qualitatively altered, diminished or enhanced rates of drug metabolism. As a consequence, polymorphisms can lead to qualitative and/or quantitative alterations in the metabolism of drugs and could be responsible for the development of a number of unexpected adverse drug reactions and host-specific susceptibility to drugs or other chemicals. An association between polymorphisms and increased toxicity risk or cancer has been reported (66). Among P450 polymorphisms, those affecting CYP2C9, CYP2C19 and, particularly CYP2D6, have the highest impact on drug metabolism (67, 68). At present, a high number of CYP2D6 allelic variants (ca. 50) have been identified, some resulting in appreciable changes in enzyme function. In contrast, CYP1A1, CYP2E1 and CYP3A4 genes are relatively well conserved and only a few, if any, rare variants yielding changes in catalytic enzyme activity have been found (65).

Interindividual differences in catalytic activities cannot be exclusively attributed to genetic polymorphisms, and non-genetic factors should be considered as a leading cause of the great variability existing in drug metabolism rates. Age, gender, hormonal status, liver pathologies and drug intake can influence the P450 function. CYP3A4, the most abundant P450 in the human liver, constitutes a good example of phenotypic variability. Variations in CYP3A4 activity cannot be explained by the genotype as only rare allelic variants with no major functional effects have been identified (69). CYP3A4 variability is probably a direct consequence of the modulation of gene expression by environmental factors. The enzyme

is highly induced by different groups of compounds, including therapeutic agents such as glucocorticoids, antibiotics, anticonvulsants, or anti-inflammatories (14, 70-72).

The use of *in vitro* models able to reflect interindividual variations in drug metabolism will notably increase the relevance of preclinical metabolic studies. The analysis of P450 enzymes in microsomes from a human liver bank revealed the existence of considerable variability (73). As an example, high variations (>50-fold) were found in CYP3A4 activity levels in microsome preparations form 30 different livers (Figure 5A). A similar picture is observed when CYP3A4 activity is measured in primary human hepatocyte cultures prepared from different donors (Figure 5B). Incubation of the compound of interest with human liver microsomes or hepatocytes expressing different levels of P450 enzymes will help to anticipate information on

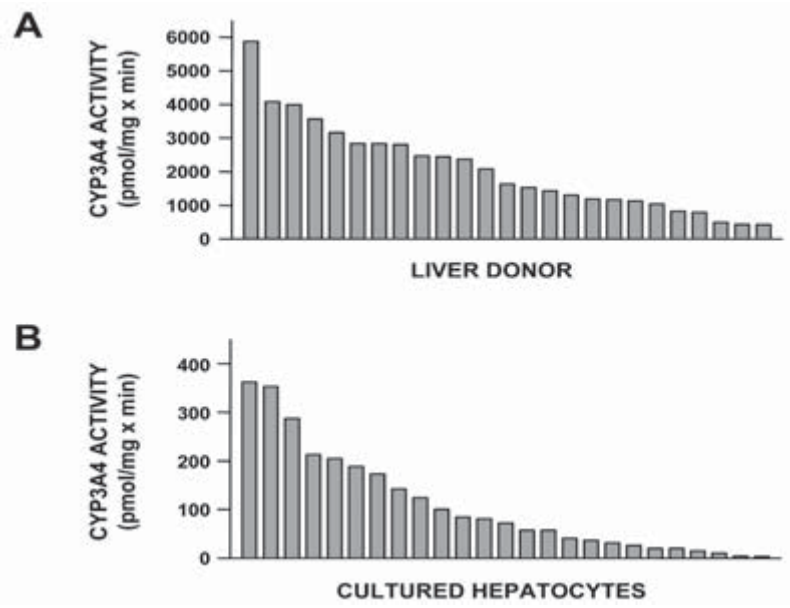

FIGURE 5. ***Variability of CYP3A4 activity in human liver and human hepatocytes from different donors.*** *CYP3A4 activity (testosterone 6β-hydroxylation) was determined (A) in microsomes from a human liver bank (n = 30), and (B) in different preparations of human hepatocytes. The individual data for CYP3A4 activity are plotted and sorted in decreasing order to show inter-individual variability.*

potential interindividual variations in metabolic rates or metabolite profile. The utility of cultured hepatocytes for this purpose was examined by investigating the metabolism of a model compound, aceclofenac, both *in vitro* and *in vivo*. The *in vitro* metabolism of the compound was studied in hepatocyte cultures obtained from human patients who, after clinical recovery, received a sub-clinical dose of the drug to examine the metabolism *in vivo* (presence of metabolites in urine) (74). A remarkable similarity between metabolic profile and the extent of aceclofenac *in vitro* and *in vivo* metabolism was found for each donor. These results support the idea that cultured human hepatocytes are a good approximation to anticipate *in vivo* metabolic profile of a drug, and back the validation process needed by this experimental model to be considered as a simplified tool to study drug metabolism and drug interactions. Moreover, the interindividual variations in drug metabolism also become evident in *in vitro* studies.

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