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Role of Human Liver Microsomes in In Vitro Metabolism of Drugs—A Review

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Abstract Drug metabolism studies are essential and necessary during the evaluation of drugs. This review discusses the in vitro human liver models to estimate the drug metabolic fates in vivo. Different approaches are provided and emphasis is placed on the potential of human liver microsomes for drug metabolism and inhibition studies. The methodology for these studies using human liver microsomes, applications of human liver microsomes, and the drugs studied by human liver microsomes are listed. Human liver microsomes represent a critical experimental model for the evaluation of drug metabolites with a high probability of clinical success.

Keywords Biotransformation · CYP450 · Drug · Human liver microsomes · In vitro metabolism study · Metabolism · Metabolites

Introduction

The development of a new therapeutic agent involves a preclinical screening stage. During this stage, the main properties of the drug candidate such as pharmacodynamic, pharmacokinetic, and toxicological properties are investigated. This preclinical investigation is based on both in vitro models and in vivo experiments in various animal species [1, 2].

Drug metabolism determines adverse drug reactions; a toxic drug can be detoxified or a nontoxic drug can be activated to a toxic metabolite by biotransformation. Generally, drug metabolism involves a phase I reaction where nonpolar molecules are converted into a polar compound or a phase II conjugation where suitable moieties such as glucuronic acid or sulfate or glycine or methyl group or acetyl group is added to the organic group [3].

Biotransformation occurs in many tissues, liver as the most important organ, but also the kidneys, intestines, lungs, brain, nasal epithelium, and skin can be involved [4]. The liver is the largest internal organ of the human body and is located between the digestive tract and

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the other parts of the body [5]. Liver is the rich source of heme-containing enzymes, CYP450, which play a major role in phase I oxidation reactions [6, 7]. CYPs are involved in the biosynthesis and metabolism of steroids, bile acids, fatty acids, prostaglandins, leukotriens, biogenic amines or retinoids, various carcinogens, and environmental pollutants. These enzymes can also transform nontoxic chemicals into reactive intermediates that are toxic or carcinogenic [8]. These enzymes sometimes cause bioactivation of drugs like Tegafur, an anticancer prodrug activated to 5-fluorouracil [9]. Phase II enzymes such as uridine diphosphoglucuronosyl transferase (UGT), glutathione S-transferase (GST), *N*-acetyl transferase (NAT), and sulfotransferase (ST) also have an important role in the detoxification and excretion of xenobiotics [4].

It is apparent that drug transporters (phase III) influence not only the therapeutic efficacy but also the absorption, distribution, and elimination of a drug [10]. The drug transporters are located in the epithelial and endothelial cells of the liver, gastrointestinal tract, blood–brain barrier, kidney, and other organs. These are responsible for the transport of most of the prescribed drugs across cellular barriers and thus for the concentration at the biotransformation site. Hence, the elucidation of the influence of drug transporters on the absorption, distribution, metabolism, and excretion of a drug is essential in the early development stage of new drugs. Thus, the objective was to provide a review on model systems with a strong predictive power for human biotransformation studies of drugs.

In vitro screening assays which include human liver-derived experimental systems represent the effective approach to estimate the human drug metabolic fates in vivo [11, 12]. Several in vitro human liver models have been developed during the past few decades [2]. They are: (1) perfused liver (2), liver slices (3), primary hepatocytes, (4) cytosol, (5) S9 fractions, (6) supersomes, (7) cell lines, (8) transgenic cell lines, and (9) microsomes.

In this review, an overview of different in vitro models, with a deep stress on human liver microsomes and the metabolic reactions and CYP enzymes involved in the metabolism of various drugs studied using human liver microsomes, is given.

All these models except primary hepatocytes, cell lines, liver slices, and isolated perfused liver require exogenous cofactors for activity. The cofactors used consist of NADPH-regenerating system (phase I oxidation) or uridine-5′-diphospho-α-D-glucuronic acid (UDPGA; phase II glucuronidation). NADPH is required for the measurement of oxidase activity catalyzed by P450s, flavin-containing monooxygenases, NADPH-P450 reductase, and many other oxidase enzymes [13].

Supersomes

Supersomes are a valuable supplement to human liver microsomes [14, 15]. Insect cells lack endogenous cytochrome P450 and uridinediphosphoglucuronosyl transferase activity. Therefore, microsomes which consist of vesicles of the hepatocyte endoplasmic reticulum of human CYP- or UGT-transfected insect cells are used as tools in human biotransformation studies. These are too called baculosomes because the insect cells are infected by baculo virus [16]. Hence, they have the added advantage of unique specification by expressing enzyme activity of one single CYP isoform.

Incubation with non-transfected supersomes will be conducted as a control always [17]. A NADPH-regenerating system has to be added as a cofactor. The CYP and UGT activity is provided by the supplier of the supersomes which are commercially available. The advantages of supersomes are that they are used to study the influence of different polymorphisms on drug metabolism patterns and to study drug–drug interactions (for, e.g.,

fluvoxamine–theophylline interaction at CYP1A2; [18]). Supersomes too possess some drawbacks; in UGT supersomes, the UGT active site is shielded behind a hydrophobic barrier, resulting in latent glucuronidations, but this can be overcome using a pore-forming agent like alamethicin [19].

Cytosol

Cytosol has not been used very often in drug biotransformation research so far [20–22]. The liver cytosolic fractions contain phase II enzymes such as glutathione S-transferase, *N*-acetyl transferase, and sulfotransferase. It is obtained by differential centrifugation of whole-liver homogenate like microsomes. Cofactors like acetyl coenzyme A (acetyl CoA), dithiothreitol and acetyl CoA regenerating system for NAT, adenosine-3′-phosphate-5′-phosphosulfate for ST, and glutathione (GT) for GST are necessary. Human liver cytosol is commercially available from different companies such as Gentest corporation (Becton Dickinson Company, Woburn, MA, USA) and Xenotech (Kansas City, KS, USA). The advantages of cytosol are that the biotransformation capacity of NAT, ST, and GST can be studied using cytosol either separately or in combination depending on the cofactors added, but only the soluble phase II enzymes present in the liver cytosol fraction can be investigated. The metabolic pathways of UGT, which is located on the endoplasmic reticulum, cannot be investigated with this model.

S9 Fractions

Liver S9 fractions have been used since the 1970s, but not as extensively as microsomes [23–25]. The liver S9 fraction contains both microsomal and cytosolic fractions. Exogenous cofactor, NADPH-regenerating system is supplied to meet the energy demand of the CYP enzymes. These are useful in the study of xenobiotic metabolism and drug interactions which represent the post-mitochondrial supernatant fraction from homogenized liver. They offer a more complete representation of the metabolic profile because they contain both phase I and phase II activities. In some cases, metabolites which are not produced by either the cytosolic fraction or the microsomal fraction alone are formed with S9 fractions. As S9 fraction contains lower enzyme activity when compared to cytosol or microsomes, it may leave unnoticed metabolites.

Cell Lines

Cell lines are used for drug metabolism studies only in enzyme-induced state and in cytotoxicity studies of drug and its metabolites. These are less popular when compared to other in vitro models because of their dedifferentiated cellular characteristics and incomplete expression of all families of metabolic enzymes. Human liver cell lines can be isolated from primary tumors of the liver parenchyma, seen after chronic hepatitis or cirrhosis [26]. The important requirement of cell lines is that they must resemble the normal physiology of human hepatocytes in vivo. Currently available human liver cell lines are Hep G2 (from hepatocellular carcinoma), BC2 (hepatoma), Hep 3B (hepatocellular carcinoma), C3A (hepatoblastoma), etc. (http://www.atcc.org). These are easy to culture and have stable enzyme concentration relatively. As these have low expression or absence of most important phase I and phase II drug-metabolizing enzymes, it is difficult to investigate the individual CYPs or other enzymes and metabolites in cell lines.

Transgenic Cell Lines

Cell lines are transfected at high efficiency using centrifugation of lysozyme-treated bacteria bearing the desired vector with the parent cells in the presence of polyethylene glycol protoplast fusion [2]. Crespi et al. [27] was the first to achieve stable expression of human CYPs in a human cell line. These cell lines have higher expression of CYP and UGT isozymes. The transgenic cell lines can be readily obtained from specialized companies such as ATCC and Gentest. These cell lines are used to generate metabolites for structure elucidation and pharmacological characterization and to assess drug–drug interactions. These possess the advantages of ease in culturing and the possibility of studying the single enzyme reactions, with disadvantages of very expensive compared to the other in vitro models and it will not reflect a complete in vivo situation as it expresses only a few isozymes.

Hepatocytes

Hepatocytes are used to study both phase I and phase II liver drug metabolism pathways [3] which reflect the heterogeneity of CYP expression in human liver [28]. Cultured hepatocytes [29, 30] and suspensions of primary hepatocytes are powerful tools to analyze the metabolic profile of a number of drugs with good in vitro–in vivo correlations [31–33]. Cultured hepatocytes are subject to a loss of liver-specific functions gradually with a decrease in CYP expression.

Cryopreserved hepatocytes retain the activity of most of phase I and phase II enzymes [34, 35] and are now available commercially [36]. A disadvantage is the lack of liver non-hepatocyte cells, which may be necessary for cofactor supply. Another disadvantage is that there may be inter-individual variation, which can be overcome by using mixtures of hepatocytes from multiple donors [2]. Applications of human hepatocytes in the development of a drug include the evaluation of metabolic stability, metabolite profiling and identification, hepatotoxic and drug–drug interaction potentials [28, 37].

Liver Slices

The incubation of liver slices in nutrient-rich media is another tool to study metabolism in vitro. Owing to impaired diffusion of nutrients and oxygen in the liver slices, the duration of activity of CYP is short. There are no commercially available human liver slices yet [2]. There is a possibility for studying the induction of CYP isoforms by new drugs. Its disadvantages include inadequate penetration of culture medium into the slices and short viability time period of 5 days.

Isolated Perfused Liver

Human liver is never used; only animal livers on small scale are used for biotransformation studies, which are regarded as correct model always for human drug biotransformation. Perfused animal liver is useful only in cases where bile secretion is of importance or when validation of other in vitro methods is required. This model has poor reproducibility, is labor-intensive, and its functional integrity is limited to 3 h [2].

Human Liver Microsomes

Human liver microsomes account for the most popular in vitro model. These are subcellular fractions derived from the endoplasmic reticulum of hepatic cells and are prepared by homogenization of liver [3] followed by differential centrifugation [38]. These are used for the evaluation of phase I oxidation by the addition of a cofactor such as NADP. They can as well be used to study glucuronidation by the addition of UDPGA. Human liver microsomal phospholipid comprises 49% phosphatidylcholine, 31% phosphatidylethanolamine, 14% phosphatidylserine and phosphatidylinositol, and 6% sphingomyelin [39]. Liver slices, liver cell lines, and primary hepatocytes can also be used for the preparation of microsomes [40, 41]. These subcellular fractions are a rich source of many drug-metabolizing enzymes like cytochrome P450s, flavin monooxygenases, carboxyl esterases and epoxide hydrolase, and UDP glucuronyl transferases. Therefore, they are widely used as an in vitro model system in order to investigate the metabolic fate of xenobiotics [42]. The influence of specific isozymes is studied using liver microsomes in the presence of specific inhibitors [43].

There are inter-individual variations in the activity of human liver microsomes, but this problem can be overcome by the application of pooled microsomes [17]. Individual human liver microsomes are useful in identifying critical CYP involved in the biotransformation of drug by correlating enzyme activity of a particular CYP, using a bank of human donors, to the metabolism of the drug. Gender-specific human liver microsomal pools are used to investigate the influence of gender on drug biotransformation. Applications of microsomes in drug discovery and development include metabolite identification, comparison of metabolism by different species, prediction of in vivo clearance [44], and reaction phenotyping [45, 46]. The advantages of human liver microsomes include: (a) low cost, simplicity in use, and easy storage; (b) one of the best characterized in vitro models for research in drug metabolism; (c) inter-individual variation can also be studied; and (d) the enzyme activity can be kept in crystal (frozen) forms for many years. The disadvantages include: (a) unsuitability for quantitative estimations of in vivo human biotransformation because of the presence of high level CYPs and UGTs and no competition with other enzymes; (b) the absence of other enzymes such as NAT, ST, GST, and cytosolic cofactors leave the metabolites unnoticed which are formed in intact liver cells; and (c) the in vitro incubation conditions used such as the ionic strength, pH value of the incubation medium, and the effect of organic solvents used can affect the outcomes of microsomal studies.

Table 1 shows the biotransformation of some drugs studied using human liver microsomes.

General Methodology for the Determination of Metabolites in Human Liver Microsomes

Microsomes are prepared by differential centrifugation [69] and stored at −80 °C until use [125, 153]. Microsomal protein concentration is determined by different methods using folin phenol reagent [167] or Coomassie Brilliant Blue G-2520 dye [168], etc. CYP 450 content is determined by Omura and Sato [169] method. The microsomes are incubated with the drug and incubation mixture consists of liver microsomes, test compound, NADPH generating system (usually NADP + glucose-6-phosphate dehydrogenase) [69, 106, 153], and buffers like phosphate buffer or sodium phosphate buffer [153]. The reaction is initiated by adding NADPH generating system. Then, it is incubated at 37 °C for different time points (10, 25, 40, 60 min, etc.) on a shaking water bath [153] or shaking incubator [170].

Table 1 Selected examples of drugs showing biotransformation using human liver microsomes.

S. no.	Name of drug	Category	Metabolic reaction	CYP isoform involved	Inhibitors used	Reference
1	Adinazolam	Anticonvulsant, anxiolytic	*N*-demethylation	3A4	Ketoconazole; troleandomycin	[47]
2	AF-5	Antianxiety	Hydroxylation	–	–	[48]
3	Alachlor	Pre-emergent herbicide	Oxidation	3A4	–	[49]
4	6-Amino chrysene	Promutagen (bioflavonoid)	*N*-hydroxylation	2B6, 3A4	Pentachlorophenol	[50]
5	Amiodarone	Antiarrhythmic	*N*-deethylation	3A4	Ketoconazole	[51]
6	Amitriptyline	Tricyclic antidepressant	*N*-demethylation	2C19, 3A4	Ketoconazole	[52, 53]
7	AMMC (3-(2-(*N*,*N*-diethyl-*N*-methylammonium)-ethyl)-7-methoxy-4-methyl coumarin)	Anticoagulant	Hydroxylation	2D6	CYP 2D6 antibody	[54]
8	Amprenavir	Antiretroviral	*N*-dealkylation	3A4	Ketoconazole; terfenadine; astemizole; ritonavir	[55, 56]
9	Azelastine	Antiallergy and antiasthmatic	Demethylation	3A4, 2D6, 1A2	Ketoconazole; erythromycin; fluvoxamine	[57]
10	Benzo (a) pyrene	Polycyclic hydrocarbon (carcinogen)	3-Hydroxylation	3A4, 2C8	Gestodine; troleandomycin; sulfaphenazole	[58]
11	BFBFC (5-bis(trifloro methyl)-7-benzyloxy-4-trifluoro methyl coumarin	Anticoagulant	Hydroxylation	3A4	Troleandomycin; ketoconazole	[59]
12	BFC (7-benzyloxy-4-trifluoro methyl-coumarin)	Anticoagulant	Hydroxylation	1A2, 3A4, 2C9, 2C19	Furafylline; troleandomycin	[60]
13	(+)-Bufuralol	Antihypertensive	1′-Hydroxylation; 4- and 6-hydroxylation	2D6, 1A2	Quinidine	[61, 62]
14	BVT. 2938	5 HT 2C- receptor agonist	Hydroxylation	2D6, 1A1	Quinidine	[63]

15	BYZX	Centrally acting cholinesterase inhibitor	*N*-dealkylation	3A4	Ketoconazole; cyclosporine A	[64]
16	Celecoxib	NSAID	Methyl hydroxylation	2C9, 3A4	Sulfaphenazole; ketoconazole	[65]
17	Cerivastatin	Cholesterol lowering drug	Demethylation; hydroxylation	3A	Troleandomycin	[66]
18	Chlorpromazine	Antipsychotic	7-Hydroxylation	2D6, 1A2	Quinidine; α-naphthoflavone	[67]
19	Chlorpyrifos	Organophosphothioate insecticide	Desulfuration; dearylation	2B6, 2C19	–	[68]
20	Cilnidipine	Antihypertensive	Dehydrogenation; demethylation	3A	Ketoconazole	[69]
21	Citalopram	Antidepressant	*N*–demethylation; *N*-oxidation	3A4, 2C19, 2D6	–	[70, 71]
22	Colchicine	Antigout	Demethylation	3A4	Gestodene; troleandomycin	[72]
23	Cyclophosphamide	Anticancer	4-Hydroxylation	2C9, 3A4/5	Orphenadrine	[73]
24	Desogestrel	Oral contraceptive	Hydroxylation	2C9, 2C19	Sulfaphenazole; *S*-mephenytoin	[74]
25	Dexfenfluramine	Appetite suppressant	*N*-dealkylation	2D6, 1A2	Quinidine; 7, 8-naphthoflavone	[75]
26	Dextromethorphan	Antitussive	*O*-demethylation; *N*-demethylation	3A4, 2D6	Quinidine	[76–78]
27	Diazepam	Sedative, hypnotic	Temazepam; *N*-desmethyl diazepam	3A	Troleandomycin	[79]
28	Diazinon	Organophosphorothioate insecticide	Desulfuration; dearylation	2C19, 1A2, 2B6, 3A4	–	[68]
29	Disopyramide	Antiarrhythmic	Mono-*N*-dealkylation	3A4	Anti human CYP 3A serum	[80]
30	Docetaxel	Anticancer	Oxidation	3A	Erythromycin; ketoconazole; nifedipine; midazolam; troleandomycin	[81]
31	Doxorubicin	Cytotoxic	–	3A	SKF-525A; cimetidine	[82]

Table 1 (continued)

S. no.	Name of drug	Category	Metabolic reaction	CYP isoform involved	Inhibitors used	Reference
32	Ebastine	Non-sedative; antihistamine	Desbutyro phenone; hydroxyl ebastine	3A4	Ketoconazole; troleandomycin; cyclosporine A; Midazolam	[83]
33	Endosulfan-alpha	Pesticide	Endosulfan sulfate	2B6, 3A4	Ticlopidine; ketoconazole	[84]
34	Estradiol	Potent estrogen	Hydroxylation	1A2, 3A4	Fluvoxamine; ketoconazole	[85]
35	Estrone	Potent estrogen	Hydroxylation	1A2, 3A4	Fluvoxamine; ketoconazole	[85, 86]
36	Etoposide	Antitumor	3′-Demethylation	3A4	Ketoconazole; troleandomycin; verapamil; cyclosporine A	[87]
37	Fenchone	Flavor in foods and in perfumery	Oxidation	2A6, 2B6	Thio TEPA	[88]
38	Fentanyl	Opiod analgesic	*N*-dealkylation	3A4	–	[89, 90]
39	Flumazenil	Diagnostic drug in benzodiazepine intoxication	Flumazenil acid; *N*-desmethyl flumazenil	P450	Phenyl methyl sulfonyl fluoride (PMSF)	[91]
40	Fluoxetine	Antidepressant	*O*-dealkylation	2C19, 3A4	Omeprazole; triacetyl oleandomycin	[92]
41	Flurbiprofen	NSAID	Oxidation; conjugation	2C9	Warfarin; tolbutamide; α-naphthoflavone; erythromycin	[93]
42	Flutamide	Non-steroidal antiandrogen	Hydroxylation	1A2, 3A4, 2C19	–	[94]
43	Gallopamil	Calcium channel antagonist	*N*-dealkylation; *O*-demethylation	3A4	Ketoconazole; troleandomycin	[95]
44	Granisetron	Antiemetic	9′-Demethylation; 7-hydroxylation	3A3/4	Ketoconazole	[96]
45	Halofantrine	Antimalarial	*N*-debutylation	3A4/5	Diltiazem; erythromycin; nifedipine; cyclosporine	[97]
46	Haloperidol	In schizophrenia	*N*-dealkylation	3A4	Ketoconazole	[98]

47	Ibuprofen	NSAID	2-Hydroxylation; 3-hydroxylation	2C8/9	Sulfaphenazole; retinol; arachidonic acid	[99]
48	Indomethacin	NSAID	*O*-demethylation	2C9	Sulfaphenazole; (*S*)-warfarin; tolbutamide	[100]
49	Itraconazole	Antifungal	Hydroxylation; dealkylation	3A4	Midazolam	[101]
50	Lansoprazole	Protonpump inhibitor	Hydroxylation; sulfation	2C9	Ketoconazole	[102]
51	(+)- and (−)-Limonene	Monocyclic monoterpene	6-Hydroxylation; 7-hydroxylation	2C9, 2C19	Sulfaphenazole; flavoxamine	[103]
52	Lornoxicam	NSAID	5′-Hydroxylation	2C9	Sulfaphenazole	[104]
53	Medroxy progesterone	Anticancer	Hydroxylation	3A4	Ketoconazole	[105]
54	(−)-Menthone	Component of essential oils, tooth brushing powder	Oxidation; reduction	CYP450	–	[106]
55	Methadone	In opiod dependence	Demethylation	3A4, 2C19, 2C9	Ketoconazole; troleandomycin; sulfaphenazole; omeprazole; fluoxetine	[107]
56	Metoclopramide	Antiemetic	*N*-dealkylation	2D6, 1A2	Quinidine	[108]
57	Mianserin	Antidepressant	8-Hydroxylation; *N*-oxidation; *N*-demethylation	2D6, 1A2	Quinidine; furafylline; α-naphthoflavone; troleandomycin	[109]
58	Midazolam	Anxiolytic, anticonvulsant	Hydroxylation	2C, 3A4/5	Ketoconazole	[110, 111]
59	MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydro pyridine)	Parkinson's disease inducer	*N*-demethylation	2D6, 1A2, 3A4	Quinidine; furafylline; ketoconazole	[112]
60	Naproxen	NSAID	*O*-demethylation	2C9, 1A2	Sulfaphenazole; furafylline	[113]
61	Nefiracetam	Antidepressant	Hydroxylation	3A4	Ketoconazole; troleandomycin	[114]
62	Nicotine	Stimulant	2′-Hydroxylation	2A6	–	[115]
63	*N*-Nitroso dialkylamines	Procarcinogen	Demethylation and denitrosation	2E1	–	[116, 117]
64	*N*-Nitrosodi-*n*-propyl amine	Environmental carcinogen	Depropylation; denitrosation	2E1, 2B1	Diethyl dithiocarbamic acid; troleandomycin	[118]

Table 1 (continued)

S. no.	Name of drug	Category	Metabolic reaction	CYP isoform involved	Inhibitors used	Reference
65	Nortriptyline	Tricyclic antidepressant	Hydroxylation; demethylation	2D6, 2C19, 1A2	–	[119]
66	Omeprazole	Proton pump inhibitor	5-hydroxylation; sulfoxidation	3A4, 2C19	*S*-mephenytoin	[120]
67	Parathion	Phosphorothioate pesticide	Oxidation	3A4	Ketoconazole; triacetyl oleandomycin	[121]
68	Paroxetine	Antidepressant	Demethylenation	2D6	Quinidine; quinine	[122]
69	Perazine	Antipsychotic	*N*-demethylation and *N*-oxidation	3A4 , 2C9	Sulfaphenazole; ketoconazole; methimazole	[123]
70	Perhexiline	Antianginal	cis-OH-PHX; trans-1-OH-PHX; trans-2-OH-PHX	2D6	–	[124]
71	Perphenazine	Antipsychotic	*N*-dealkylation	1A2, 3A4, 2C19, 2D6	Furafylline; ketoconazole; fluvoxamine; quinidine	[125]
72	Phenacetin	Analgesic; antipyretic	*O*-deethylation to acetaminophen	1A2	Fluvoxamine	[126, 127]
73	Pimozide	Neuroleptic	*N*-dealkylation	3A	Ketoconazole	[128]
74	Promazine	Neuroleptic	5-sulfoxidation; N-demethylation	1A2, 3A4, 2C19	Furafylline; ketoconazole; ticlopidine	[129]
75	Propofol	I.V. anesthetic	Oxidation	2B6	Orphenadrine	[130, 131]
76	Propranolol	Antihypertensive	4-Hydroxylation	2D6, 1A2	Quinidine; furafylline	[132]
77	Quinine	Antimalarial	3-Hydroxylation	3A4, 2C19	Ketoconazole; troleandomycin	[133]

78	Riddelliine	Carcinogenic to laboratory rodents (pyrrolizidine alkaloid)	Hydroxylation	3A	Triacetyleandomycin	[134, 135]
79	Rutaecarpine	In G.I. disorders and headache	10-, 11-, 12- and 3-Hydroxylation	1A2, 2D6, 3A4	α-Naphthoflavone, quinidine, ketoconazole	[136]
80	Rosiglitazone	Anidiabetic	Hydroxylation; *N*-demethylation	2C8	13-cis retinoic acid	[137, 138]
81	Salmeterol	Antiasthmatic	Oxidation	3A	Ketoconazole	[139]
82	Sertraline	Antidepressant	*N*-demethylation	2C19, 2C9	Omeprazole; sulfaphenazole	[140]
83	Sildenafil	In treatment of male erectile dysfunction	*N*-demethylation	3A4, 2C9	Ketoconazole; sulfaphenazole	[141]
84	S-MTPPA [S-2-[4-3-methyl-2-thienyl) phenyl) propionic acid]	–	5-Hydroxylation	2C9	Sulfaphenazole	[142]
85	Tamoxifen	Antiestrogen and antitumor	4-Hydroxylation; *N*-demethylation	2D6, 2C9, 3A4	Quinidine; sulfaphenazole; ketoconazole	[143]
86	Taxol	Anticancer	Hydroxylation	3A, 2C	Orphenadrine; erythromycin; troleandomycin; testosterone; diazepam	[144]
87	Terfenadine	Antihistaminic	Oxidative *N*-dealkylation; oxidation of *tert*-butyl methyl group; oxidation	3A4	Gestodene	[145]
88	Timolol	In glaucoma treatment	Hydroxylation	2D6	Quinidine	[146]
89	Tolbutamide	Oral hypoglycemic	4-Hydroxylation	2C9, 2C19, 2C8, 2C10	Sulfaphenazole	[147–150]
90	Triazolam	Sedative	Hydroxylation	3A	Ketoconazole	[110, 151]
91	Valsartan	Angiotensin-II receptor antagonist	4-Hydroxylation	2C9	Diclofenac	[152]

Table 1 (continued)

S. no.	Name of drug	Category	Metabolic reaction	CYP isoform involved	Inhibitors used	Reference
92	Venlafaxine	Antidepressant	*O*-demethylation; *N*-demethylation	2D6, 3A4	Quinidine; ketoconazole	[153, 154]
93	(−)-Verbenone	Component of essential oil from Rosemary	10-Hydroxylation	2A6	–	[155, 156]
94	Vicriviroc	Antiretroviral	*N*-oxidation; *O*-demethylation; *N*,*N*-dealkylation ; oxidation to carboxylic acid	3A4	Ketoconazole; azamulin	[157]
95	Vinblastine	Antimitotic	Deacetyl vinblastine	3A	Ketoconazole; erythromycin; troleandomycin	[158]
96	Vindesine	Antineoplastic	–	3A	Quinidine; erythromycin; troleandomycin	[159]
97	Vinflunine	Antimitotic	*N*-oxidation or hydroxylation ; epoxidation	3A4	Ketoconazole	[160]
98	Vinorelbine	Antineoplastic	Deacetylation; dealkylation; oxidation; hydroxylation	3A4	Furafylline; diethyl dithiocarbamate; troleandomycin	[161, 162]
99	Zaleplon	Hypnotic	*N*-deethylation	3A4	Troleandomycin	[163, 164]
100	Zidovudine	Antiretroviral	AZT-glucuronide; 3′-amino-3′-deoxy thymidine	3A, 2A, 2B1	Ketoconazole; fluconazole; indinavir; ritonavir; saquinavir; metyrapone	[165]
101	Zotepine	Antipsychotic	*N*-demethylation; *S*-oxidation; 2- and 3-hydroxylation	3A4, 1A2, 2D6	Furafylline; sulfaphenazole	[166]

The reaction is terminated by adding stop reagents such as sodium hydroxide [69] or hydrochloric acid [147] or ice-cold carbonate/bicarbonate buffer [125] or cold acetonitrile [105] or by trichloroacetic acid [137], etc. to precipitate proteins. Reaction mixtures are extracted for metabolites with organic solvents and are centrifuged. Then, organic fraction is evaporated under a gentle stream of air or nitrogen gas, etc. The dried extracts are refrigerated at 4 °C before high-performance liquid chromatography (HPLC) analysis which is usually on the following day [153]. Residue obtained is dissolved in mobile phase and metabolites formed are determined by HPLC, mass spectrometry (MS), LC/MS, gas chromatography–MS, LC/nuclear magnetic resonance spectra.

Enzyme Kinetics in Drug Metabolism

In vitro characterization of drug metabolism begins with an enzyme kinetic analysis of metabolite formation rate using human liver microsomes. The two most important enzyme kinetic parameters used to analyze the data are K_m, Michaelis–Menten constant (concentration at which 50% of the maximal velocity is observed), and V_{max} (maximum reaction velocity of the enzyme). Reaction velocities (V) are expressed as nanomoles per hour per milligram protein when human liver microsomes are used and as nanomoles per hour per nanomole CYP when activities of CYP isoforms are measured. If the conversion of substrate to product is catalyzed by a single enzyme (one-enzyme model), the equation is [96]:

$$V = \frac{V_{max}[S]}{K_m + [S]}$$

where V_{max} is the maximal velocity of reaction, [S] is the substrate concentration, and v is the rate of product formation (or substrate disappearance).

When two or more CYP forms with distinct affinities may catalyze a given drug metabolism, the relationship between v and S is biphasic and may be described by a high- and a low-affinity component using a two-enzyme model: $V = \frac{V_{max}1[S]}{K_m1+[S]} + \frac{V_{max}2[S]}{K_m2+[S]}$ where K_m 1 and K_m 2 are the affinity constants for high- and low-affinity components and V_{max} 1 and V_{max} 2 are the maximum enzyme velocities for the high- and low-affinity components, respectively.

Nowadays, different softwares are used for calculating the parameters from untransformed data using the Michaelis–Menten equation [106]. This gives more accurate and more reproducible values than the graphical method.

F test indicates whether a one- or two-binding-site model is optimal. An Eadie–Hofstee plot, V vs V/S (where S is substrate concentration), is used to illustrate multiple binding sites [171, 172]. A Student's t test is used to test for statistical significance [125]. Spearman rank test is used to determine correlations among various enzyme activities [147].

Measures of Metabolism

Measures of metabolism include metabolic stability of a drug, metabolite and metabolic route identification, identification of CYPs metabolizing a drug, utilization of CYP-

selective chemical inhibitors, and CYP-specific antibodies correlation analysis. The metabolic stability of a new drug, under developmental stage, determines its future as a drug candidate. If a drug is metabolized rapidly in human liver preparations, its bioavailability in vivo is most probably too low for it to be a drug candidate. This depends on the route of administration of the drug. By determining the concentration and time dependence of formation of a metabolite from a new drug on the disappearance of the drug in vitro in an appropriate system, its metabolic fate and half-life in vivo can be predicted. Such similar studies performed in human and test species give valuable information in the selection of test species for pharmacokinetic and toxicological studies. Metabolite identification is developed from incubation with human liver microsomes [106]. By employing LC/MS methods, it is possible to determine the exact molecular masses and metabolic structures with high accuracy. After the characterization of metabolic stability and metabolic route, the in vivo prediction requires clarification of drug-metabolizing enzymes which participate in the in vitro biotransformation of the drug. After determining enzyme kinetic parameters, the CYPs involved in the drug metabolism can be characterized by either chemical or antibody inhibitors selective or specific for respective CYPs. The effect of chemical inhibitors is investigated by incubating human liver microsomes with test compound in the presence of various P450 isoform-specific inhibitors [137]. The reaction is initiated by the addition of NADPH generating system. Many compounds are selective for only the desired enzyme at relatively low concentration. CYP3A4 is inhibited by ketoconazole which is the most widely used [47, 51, 69], quinidine for 2D6 [67, 76], and furafylline for 1A2 [112, 125, 132]; 2C9 is selectively inhibited by sulfaphenazole [65, 123], pyridine for 2E1 [173], etc. The inhibitory effects of these inhibitors on the metabolism of the drug would reflect which P450 isoform is involved in the metabolism [3]. The inhibitory potential is investigated by performing assays for specific cytochrome P450 activity in the absence or presence of drug candidate using microsomes. IC_{50} values and inhibition constant, K_i, are determined. The IC_{50} value is defined as a concentration of inhibitor that causes 50% inhibition of an original enzyme activity. The IC_{50} value can be determined by analyzing the relationship between inhibitor concentration and decrement in reaction velocity performed at a fixed substrate concentration. The inhibitory constant (IC_{50}) is calculated using unweighted data from the equation [97] that follows:

$$v = \frac{V_0}{1 + \left[\frac{[I]}{IC_{50}}\right]^S}$$

where V_0 is the uninhibited velocity, v the observed velocity, $[I]$ the inhibitor concentration, and S the slope factor.

Another constant, K_i, is related to the affinity of a compound to an enzyme, which is estimated either graphically by using plotting methods or by nonlinear regression analysis (e.g., Dixon plot, Lineweaver–Burk plot, etc.). Various factors affect the accuracy of the estimation of K_i such as incubation conditions, non-specific binding to microsomal proteins, chemical inhibitors used, etc. [174]. The values of IC_{50} and K_i are determined by taking the concentration of marker substrates around their corresponding K_m values and about 0.5- to 4-fold of their corresponding K_m values, respectively. Alternatively, the inhibition of enzyme activity is expressed as percent of control activities. Inhibition results have been expressed as the mean ± SD when appropriate [96]. Antibodies are used if there are no sufficient specific chemical inhibitors available for a certain enzyme (e.g.,

CYP2B6 [175]). In correlation analysis, the measured CYP-specific activities are correlated against the rate of the metabolic pathway of a drug in individual liver sample. Another approach is to correlate the levels of an individual CYP determined by Western blot analysis against the drug activity [176, 177]. The higher the correlation between the activities, the larger is the likelihood that the respective CYP enzyme is responsible for the drug metabolism. The values of correlation coefficient (r) may range from −1 to 1, with 0 referring to an absent correlation and 1 to a perfect positive correlation between the two activities.

Table 2 shows marker reactions for particular CYP isoform for correlation studies which can be employed as quality criteria of the metabolic capability.

Conclusion

The metabolite profile of a drug obtained in vitro generally reflects the in vivo metabolite pattern, although limited to qualitative aspects. From physiological and biochemical points of view, human liver microsomes are useful to obtain the in vitro metabolite profile of a drug. Human liver microsomes are the subcellular fractions derived from the endoplasmic reticulum of hepatic cells and are a rich source of many drug-metabolizing enzymes like cytochrome P450s, flavin monooxygenases, carboxyl esterases, and epoxide hydrolase. Therefore, this system better simulates the in vivo situation. The present review suggests that the overall metabolism of most of the drugs appears to be mainly catalyzed by CYPs 3A4, 2C19, 2C9, 2D6, 1A2, and, to a less extent, comparatively by CYPs 2B6, 2C8, 1A1, 2A6, 2E1, 2B1, and 2C10 in human liver microsomes. The identification of drug-metabolizing enzymes involved in the major metabolic pathways of a compound helps in predicting the probable drug–drug interactions in humans. The different in vitro techniques used to study drug metabolism in humans become increasingly important in the early development stages of a new drug before starting in vivo experiments so that the most promising drugs are selected and in vivo testing can be performed as efficiently as possible. Human liver microsomes represent a critical experimental model for drug development, thus allowing early evaluation of human drug properties to guide the design and selection of drug candidates with a high probability of clinical success.

Table 2 Marker reactions showing CYP isoform involved in correlation studies.

S. no.	CYP	Marker reaction
1	1A2	7-Ethoxy resorufin o-deethylation [97]
2	2A6	Coumarin 7-hydroxylation [97]
3	2C8	Paclitaxel 6a-hydroxylation [138]
4	2C9	Diclofenac 4′-hydroxylation [153]
5	2C19	S-mephenytoin 4′-hydroxylation [163]
6	2D6	Bufuralol 1′-hydroxylation [54, 178]
7	2E1	Chlorzoxazone 6-hydroxylation [163]
8	3A4	Testosterone 6β-hydroxylation, Nifedipine oxidation [133, 145, 178]

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References

1. Koster, A. S., de Mol, N. J., Storm, G., van Heuven-Nolsen, D., van den Brink, G., & Perquin, J. (1997). *Introduction to pharmacogenesis*. Utrecht: Faculty of Pharmaceutical Sciences.
2. Brandon, E. F. A., Raap, C. D., Meijerman, I., Beijnen, J. H., & Schellens, J. H. M. (2003). An update on in vitro test methods in human hepatic drug biotransformation research: Pros and cons. *Toxicology and Applied Pharmacology, 189*, 233–246.
3. Li, A. P. (2005). Preclinical *in vitro* screening assays for drug-like properties. *Drug Discovery Today: Technologies, 2*, 179–185.
4. Lu, F. C. (1996). *Basic toxicology: Fundamentals, target organs and risk assessment* (3rd ed., pp. 27–39). Washington: Taylor and Francis.
5. Moffett, D. F., Moffett, S. B., & Schauf, C. L. (1993). *Human physiology.* St. Louis, MO: Mosby.
6. Derelanko, M. J., & Hollinger, M. A. (1995). In M. J. Derelanko & M. A. Hollinger (Eds.), *Handbook of toxicology, 1st ed* (pp. 539–579). New York: CRC.
7. Rang, H. P., Dale, M. M., & Ritter, J. M. (1996). *Pharmacology.* London: Churchill Livingstone.
8. Rendic, S., & Di Carlo, F. J. (1997). Human cytochrome P450 enzymes: A status report summarizing their reactions, substrates, inducers and inhibitors. *Drug Metabolism Reviews, 29*, 413–580.
9. Komatsu, T., Yamazaki, H., Shimada, N., Nakajima, M., & Tsuyoshi, Y. (2000). Roles of cytochromes P450 1A2, 2A6 and 2C8 in 5-fluoro uracil formation from Tegafur, an anticancer prodrug, in human liver microsomes. *Drug Metabolism and Disposition: The Biological Fate of Chemicals, 28*, 1457–1463.
10. Zhang, Y., Bachmeier, C., & Miller, D. W. (2003). *In vitro* and *in vivo* models for assessing drug efflux transporter activity. *Advanced Drug Delivery Reviews, 55*, 31–51.
11. Li, A. P. (2004). *In vitro* approaches to evaluate ADMET drug properties. *Current Topics in Medicinal Chemistry, 4*, 701–706.
12. Li, A. P. (2001). Screening for human ADME/Tox drug properties in drug discovery. *Drug Discovery Today, 6*, 357–366.
13. Taavitsainen, P., Anttila, M., Nyman, L., Karnani, H., Salonen, J. S., & Pelkonen, O. (2000). Selegiline metabolism and cytochrome P450 enzymes: In vitro study in human liver microsomes. *Pharmacology and Toxicology, 86*, 215–221.
14. Huang, Z., Roy, P., & Waxman, D. J. (2000). Role of human liver microsomal CYP3A4 and CYP2B6 in catalyzing N-dichloroethylation of cyclophosphamide and ifosfamide. *Biochemical Pharmacology, 59*, 961–972.
15. Rendic, S., Nolteernsting, E., & Schänzer, W. (1999). Metabolism of anabolic steroids by recombinant human cytochrome P450 enzymes: Gas chromatographic–mass spectrometric determination of metabolites. *Journal of Chromatography B, Biomedical Sciences and Applications, 735*, 73–83.
16. Chen, L., Buters, J. T., Hardwick, J. P., Tamura, S., Penman, B. W., Gonzalez, F. J., et al. (1997). Coexpression of cytochrome P450 2A6 and human NADPH-P450 oxido reductase in the baculo virus system. *Drug Metabolism and Disposition, 25*, 399–405.
17. Araya, Z., & Wikvall, K. (1999). 6α-Hydroxylation of taurochenodeoxycholic acid and lithocholic acid by CYP3A4 in human liver microsomes. *Biochimica et Biophysica Acta, 1438*, 47–54.
18. Yao, C., Kunze, K. L., Kharasch, E. D., Wang, Y., Trager, W. F., Ragueneau, I., et al. (2001). Fluvoxamine–theophylline interaction: Gap between in vitro and in vivo constants toward cytochrome P450 1A2. *Clinical Pharmacology and Therapeutics, 70*, 415–224.
19. Fisher, M. B., Paine, M. F., Strelevitz, T. J., & Wrighton, S. A. (2001). The role of hepatic and extrahepatic UDP-glucuronosyl transferases in human drug metabolism. *Drug Metabolism Reviews, 33*, 273–297.
20. Favetta, P., Guitton, J., Degoute, C. S., Van Daele, L., & Boulieu, R. (2000). High performance liquid chromatographic assay to detect hydroxylate and conjugate metabolites of propofol in human urine. *Journal of Chromatography B, Biomedical Sciences and Applications, 742*, 25–35.
21. Frandsen, H., & Alexander, J. (2000). *N*-acetyl transferase-dependent activation of 2-hydroxy amino-1-methyl-6-phenyl imidazo[4,5-*b*]pyridine: formation of 2-amino-1-methyl-6-(5-hydroxy)phenyl imidazo (4,5-*b*)pyridine, a possible biomarker for the reactive dose of 2-amino-1-methyl-6-phenyl imidazo(4,5-*b*)pyridine. *Carcinogenesis, 21*, 1197–1203.

22. Long, L., Moschel, R. C., & Dolan, M. E. (2001). Debenzylation of O^6-benzyl-8-oxoguanine in human liver: Implications for O^6-benzylguanine metabolism. *Biochemical Pharmacology, 61*, 721–726.
23. Mae, T., Inaba, T., Konishi, E., Hosoe, K., & Hidaka, T. (2000). Identification of enzymes responsible for rifalazil metabolism in human liver microsomes. *Xenobiotica, 30*, 565–574.
24. Mandagere, A. K., Thompson, T. N., & Hwang, K. K. (2002). Graphical model for estimating oral bioavailability of drugs in humans and other species from their Caco-2 permeability and *in vitro* liver enzyme metabolic stability rates. *Journal of Medicinal Chemistry, 45*, 304–311.
25. Sumida, K., Ooe, N., Nagahori, H., Saito, K., Isobe, N., Kaneko, H., et al. (2001). An *in vitro* reporter gene assay method incorporating metabolic activation with human and rat S9 or liver microsomes. *Biochemical and Biophysical Research Communications, 280*, 85–91.
26. Crommelin, D. J. A., & Sindelar, R. D. (1997). *Pharmaceutical biotechnology: An introduction for pharmacists and pharmaceutical scientists*. New York: Harwood.
27. Crespi, C. L., Langenbach, R., & Penman, B. W. (1993). Human cell lines, derived from AHH-1 TK+/− human lymphoblasts, genetically engineered for expression of cytochromes P450. *Toxicology, 82*, 89–104.
28. Gomez-Lechon, M. J., Donato, M. T., Castell, J. V., & Jover, R. (2004). Human hepatocytes in primary culture: The choice to investigate drug metabolism in man. *Current Drug Metabolism, 5*, 443–462.
29. Chenery, R. J., Ayrton, A., Oldham, H. G., Standring, P., Norman, S. J., Seddon, T., et al. (1987). Diazepam metabolism in cultured hepatocytes from rat, rabbit, dog, guinea pig and man. *Drug Metabolism and Disposition, 15*, 312–317.
30. Le Bigot, J. F., Begue, J. M., Kiechel, J. R., & Guillouzo, A. (1987). Species differences in metabolism of ketotifen in rat, rabbit and man: Demonstration of similar pathways in vivo and in cultured hepatocytes. *Life Sciences, 40*, 883–890.
31. Berry, M. N., Halls, H. J., & Grivell, M. B. (1992). Techniques for pharmacological and toxicological studies with isolated hepatocyte suspensions. *Life Sciences, 51*, 1–16.
32. Cross, D. M., Bell, J. A., & Wilson, K. (1995). Kinetics of ranitidine metabolism in dog and rat isolated hepatocytes. *Xenobiotica, 25*, 367–375.
33. Bayliss, M. K., Bell, J. A., Jenner, W. N., Park, G. R., & Wilson, K. (1999). Utility of hepatocytes to model species differences in the metabolism of loxtidine and to predict pharmacokinetic parameters in rat, dog and man. *Xenobiotica, 29*, 253–268.
34. Annaert, P. P., Turncliff, R. Z., Booth, C. L., Thakker, D. R., & Brouwer, K. L. (2001). P-glycoprotein-mediated in vitro biliary excretion in sandwich-cultured rat hepatocytes. *Drug Metabolism and Disposition, 29*, 1277–1283.
35. Silva, J. M., Day, S. H., & Nicoll-Griffith, D. A. (1999). Induction of cytochrome P450 in cyropreserved rat and human hepatocytes. *Chemico-Biological Interactions, 121*, 49–63.
36. Hengstler, J. G., Utesch, D., Steinberg, P., Platt, K. L., Diener, B., Ringel, M., et al. (2000). Cryopreserved primary hepatocytes as a constantly available in vitro model for the evaluation of human and animal drug metabolism and enzyme induction. *Drug Metabolism Reviews, 32*, 81–118.
37. Li, A. P. (2007). Human hepatocytes: Isolation, cryopreservation and applications in drug development. *Chemico-Biological Interactions, 168*, 16–29.
38. Pelkonen, O., Kaltiala, E. H., Larmi, T. K., & Karki, N. T. (1974). Cytochrome P450 linked monooxygenase system and drug-induced spectral interactions in human liver microsomes. *Chemico-Biological Interactions, 9*, 205–216.
39. Waskell, L., Koblin, D., & Canova-Davis, E. (1982). The lipid composition of human liver microsomes. *Lipids, 17*, 317–320.
40. Olsen, A. K., Hansen, K. T., & Friis, C. (1997). Pig hepatocytes as an *in vitro* model to study the regulation of human CYP3A4: Prediction of drug-drug interactions with 17α-ethynyl estradiol. *Chemico-Biological Interactions, 107*, 93–108.
41. Skaanild, M. T., & Friis, C. (2000). Expression changes of CYP2A and CYP3A in microsomes from pig liver and cultured hepatocytes. *Pharmacology and Toxicology, 87*, 174–178.
42. Eichelbaum, M., & Burk, O. (2001). CYP3A genetics in drug metabolism. *Nature Medicine, 7*, 285–287.
43. Birkett, D. J., Mackenzie, P. I., Veronese, M. E., & Miners, J. O. (1993). *In vitro* approaches can predict human drug metabolism. *Trends in Pharmacological Sciences, 14*, 292–294.
44. Iwatsubo, T., Hirota, N., Ooie, T., Suzuki, H., Shimada, N., Chiba, K., et al. (1997). Prediction of *in vivo* drug metabolism in the human liver from *in vitro* metabolism data. *Pharmacology and Therapeutics, 73*, 147–171.
45. Guengerich, F. P. (1989). In A. W. Hayes (Ed.), *Principles and methods of toxicology* (pp. 777–814). New York: Raven.
46. Woolf, T. F. (1999). *Hand book of drug metabolism*. New York: Marcel Dekker.

47. Venkatakrishnan, K., von Moltke, L. L., Duan, S. X., Fleishaker, J. C., Shader, R. I., & Greenblatt, D. J. (1998). Kinetic characterization and identification of the enzymes responsible for the hepatic biotransformation of adinazolam and *N*-desmethyl adinazolam in man. *Journal of Pharmacy and Pharmacology, 50*, 265–274.
48. Li, N., Zhang, J. L., & Zhou, T. H. (2001). *In vitro* metabolic studies of the novel anti anxietic drug AF-5 and its metabolites in human liver microsome incubation system. *Acta Pharmaceutica Sinica, 36*, 528–531.
49. Coleman, S., Liu, S., Linderman, R., Hodgson, E., & Rose, R. L. (1999). *In vitro* metabolism of alachlor by human liver microsomes and human cytochrome P450 isoforms. *Chemico-Biological Interactions, 122*, 27–39.
50. Yamazaki, H., Mimura, M., Oda, Y., Inui, Y., Shiraga, T., Iwasaki, K., et al. (1993). Roles of different forms of cytochrome P450 in the activation of the promutagen 6-amino chrysene to genotoxic metabolites in human liver microsomes. *Carcinogenesis, 14*, 1271–1278.
51. Fabre, G., Juhan, B., Saint-Aubert, B., Joyeux, H., & Berger, Y. (1993). Evidence for CYP3A-mediated *N*-deethylation of amiodarone in human liver microsomal fractions. *Drug Metabolism and Disposition, 21*, 978–985.
52. Venkatakrishnan, K., Greenblatt, D. J., von Moltke, L. L., Schmider, J., Harmatz, J. S., & Shader, R. I. (1998). Five distinct human cytochromes mediate amitriptyline *N*-demethylation in vitro: Dominance of CYP2C19 and 3A4. *Journal of Pharmacy and Pharmacology, 38*, 112–121.
53. Venkatakrishnan, K., von Moltke, L. L., Obach, R. S., & Greenblatt, D. J. (2000). Microsomal binding of amitriptyline: Effect on estimation of enzyme kinetic parameters in vitro. *Journal of Pharmacology and Experimental Therapeutics, 293*, 343–350.
54. Chauret, N., Dobbs, B., Lackman, R. L., Bateman, K., Nicoll-Griffith, D. A., Stresser, D. M., et al. (2001). The use of 3-[2-*N*,*N*-diethyl-*N*-methyl ammonium)ethyl]-7-methoxy-4-methyl coumarin (AMMC) as a specific CYP2D6 probe in human liver microsomes. *Drug Metabolism and Disposition, 29*, 1196–1200.
55. Decker, C. J., Laitinen, L. M., Bridson, G. W., Raybuck, S. A., Tung, R. D., & Chaturvedi, P. R. (1998). Metabolism of amprenavir in liver microsomes: Role of CYP3A4 inhibition for drug interactions. *Journal of Pharmaceutical Sciences, 87*, 803–807.
56. Treluyer, J. M., Bowers, G., Cazali, N., Sonnier, M., Rey, E., Pons, G., et al. (2003). Oxidative metabolism of amprenavir in the human liver. Effect of CYP 3A maturation. *Drug Metabolism and Disposition, 31*, 275–281.
57. Nakajima, M., Nakamura, S., Tokudome, S., Shimada, N., Yamazaki, H., & Yokoi, T. (1999). Azelastine *N*-demethylation by cytochrome P-450 (CYP) 3A4, CYP 2D6 and CYP1A2 in human liver microsomes: Evaluation of approach to predict the contribution of multiple CYPs. *Drug Metabolism and Disposition, 27*, 1381–1391.
58. Yun, C. H., Shimada, T., & Guengerich, F. P. (1992). Roles of human liver cytochrome P450 2C and 3A enzymes in the 3-hydroxylation of benzo(*a*)pyrene. *Cancer Research, 52*, 1868–1874.
59. Renwick, A. B., Lewis, D. F., Fulford, S., Surry, D., Williams, B., Worboys, P. D., et al. (2001). Metabolism of 2, 5-bis(trifluoro methyl)-7-benzyloxy-4-trifluoro methyl coumarin by human hepatic CYP isoforms: Evidence for selectivity towards CYP3A4. *Xenobiotica, 31*, 187–204.
60. Renwick, A. B., Surry, D., Price, R. J., Lake, B. G., & Evans, D. C. (2000). Metabolism of 7-benzyloxy-4-trifluoromethyl-coumarin by human hepatic cytochrome P450 isoforms. *Xenobiotica, 30*, 955–969.
61. Mankowski, D. C. (1999). The role of CYP2C19 in the metabolism of (+/−) Bufuralol, prototypic substrate of CYP2D6. *Drug Metabolism and Disposition, 27*, 1024–1028.
62. Yamazaki, H., Guo, Z., Persmark, M., Mimura, M., Inoue, K., Guengerich, F. P., et al. (1994). Bufuralol hydroxylation by cytochrome P450 2D6 and 1A2 enzymes in human liver microsomes. *Molecular Pharmacology, 46*, 568–577.
63. Baranczewski, P., Edlund, P. O., & Postlind, H. (2006). Characterization of the cytochrome P450 enzymes and enzyme kinetic parameters for metabolism of BVT.2938 using different in vitro systems. *Journal of Pharmaceutical and Biomedical Analysis, 40*, 1121–1130.
64. Xia, Z. L., Ying, J. Y., Sheng, R., Zeng, S., Hu, Y. Z., & Yao, T. W. (2007). *In vitro* metabolism of BYZX in human liver microsomes and the structural elucidation of metabolite by liquid chromatography–mass spectrometry method. *Journal of Chromatography B, Analytical Technologies in the Biomedical and Life Sciences, 857*, 266–274.
65. Tang, C., Shou, M., Mei, Q., Rushmore, T. H., & Rodrigues, A. D. (2000). Major role of human liver microsomal cytochrome P450 2C9 (CYP2C9) in the oxidative metabolism of celecoxib, a novel cyclooxygenase-II inhibitor. *Journal of Pharmacology and Experimental Therapeutics, 293*, 453–459.

66. Boberg, M., Angerbauer, R., Fey, P., Kanhai, W. K., Karl, W., Kern, A., et al. (1997). Metabolism of cerivastatin by human liver microsomes in vitro: Characterization of primary metabolic pathways and of cytochrome P450 isozymes involved. *Drug Metabolism and Disposition: the Biological Fate of Chemicals, 25*, 321–331.
67. Yoshii, K., Kobayashi, K., Tsumuji, M., Tani, M., Shimada, N., & Chiba, K. (2000). Identification of human cytochrome P450 isoforms involved in the 7-hydroxylation of chlorpromazine by human liver microsomes. *Life Sciences, 67*, 175–184.
68. Sams, C., Cocker, J., & Lennard, M. S. (2004). Biotransformation of chlorpyrifos and diazinon by human liver microsomes and recombinant human cytochrome P450s (CYP). *Xenobiotica, 34*, 861–873.
69. Liu, X.-Q., Zhao, Y., Dan, L. I., Qian, Z.-Y., & Wang, G.-J. (2003). Metabolism and metabolic inhibition of cilnidipine in human liver microsomes. *Acta Pharmacologica Sinica, 24*, 263–268.
70. Rochat, B., Amey, M., Gillet, M., Meyer, U. A., & Baumann, P. (1997). Identification of three cytochrome P450 isozymes involved in *N*-demethylation of citalopram enantiomers in human liver microsomes. *Pharmacogenetics, 7*, 1–10.
71. Olesen, O. V., & Linnet, K. (1999). Studies on the stereo selective metabolism of citalopram by human liver microsomes and cDNA-expressed cytochrome P450 enzymes. *Pharmacology, 59*, 298–309.
72. Tateishi, T., Soucek, P., Caraco, Y., Guengerich, F. P., & Wood, A. J. (1997). Colchicine biotransformation by human liver microsomes. Identification of CYP3A4 as the major isoform responsible for colchicines demethylation. *Biochemical Pharmacology, 53*, 111–116.
73. Ren, S., Yang, J.-S., Kalhorn, T. F., & Slattery, J. T. (1997). Oxidation of cyclophosphamide to 4-hydroxy cyclophosphamide and deschloroethyl cyclophosphamide in human liver microsomes. *Cancer Research, 57*, 4229–4235.
74. Gentile, D. M., Verhoeven, C. H., Shimada, T., & Back, D. J. (1998). The role of CYP2C in the in vitro bioactivation of the contraceptive steroid desogestrel. *Journal of Pharmacology and Experimental Therapeutics, 287*, 975–982.
75. Haritos, V. S., Ching, M. S., Ghabrial, H., Gross, A. S., Taavitsainen, P., Pelkonen, O., et al. (1998). Metabolism of dexfenfluramine in human liver microsomes and by recombinant enzymes: Role of CYP2D6 and 1A2. *Pharmacogenetics, 8*, 423–432.
76. von Moltke, L. L., Greenblatt, D. J., Grassi, J. M., Granda, B. W., Venkatakrishnan, K., Shmider, J., et al. (1998). Multiple human cytochromes contribute to biotransformation of dextromethorphan in vitro: Role of CYP2C9, CYP2C19, CYP2D6 and CYP3A. *Journal of Pharmacy and Pharmacology, 50*, 997–1004.
77. Schmider, J., Greenblatt, D. J., Fogelman, S. M., von Moltke, L. L., & Shader, R. I. (1997). Metabolism of dextromethorphan in vitro: Involvement of cytochromes P450 2D6 and 3A3/4, with a possible role of 2E1. *Biopharmaceutics & Drug Disposition, 18*, 227–240.
78. Kerry, N. L., Somogyi, A. A., Bochner, F., & Mikus, G. (1994). The role of CYP2D6 in primary and secondary oxidative metabolism of dextromethorphan: In vitro studies using human liver microsomes. *British Journal of Clinical Pharmacology, 38*, 243–248.
79. Andersson, T., Miners, J. O., Veronese, M. E., & Birkett, D. J. (1994). Diazepam metabolism by human liver microsomes is mediated by both S-mephenytoin hydroxylase and CYP3A isoforms. *British Journal of Clinical Pharmacology, 38*, 131–137.
80. Echizen, H., Tanizaki, M., Tatsuno, J., Chiba, K., Berwick, T., Tani, M., et al. (2000). Identification of CYP3A4 as the enzyme involved in the mono-*N*-dealkylation of disopyramide enantiomers in humans. *Drug Metabolism and Disposition: The Biological Fate of Chemicals, 28*, 937–944.
81. Marre, F., Sanderink, G. J., de Sousa, G., Gaillard, C., Martinet, M., & Rahmani, R. (1996). Hepatic biotransformation of docetaxel (Taxotere) in vitro: Involvement of the CYP3A subfamily in humans. *Cancer Research, 56*, 1296–1302.
82. Lewis, A. D., Lau, D. H. M., Duran, G. E., Wolf, C. R., & Sikic, B. I. (1992). Role of cytochrome P450 from the human CYP3A gene family in the potentiation of morpholino doxorubicin by human liver microsomes. *Cancer Research, 52*, 4379–4384.
83. Hashizume, T., Mise, M., Terauchi, Y., Luan, O., Fujii, T., Miyazaki, H., et al. (1998). *N*-dealkylation and hydroxylation of ebastine by human liver cytochrome P450. *Drug Metabolism and Disposition: The Biological Fate of Chemicals, 26*, 566–571.
84. Casabar, R. C., Wallace, A. D., Hodgson, E., & Rose, R. L. (2006). Metabolism of endosulfan-alpha by human liver microsomes and its utility as a simultaneous in vitro probe for CYP2B6 and CYP3A4. *Drug Metabolism and Disposition: The Biological Fate of Chemicals, 34*, 1779–1785.
85. Yamazaki, H., Shaw, P. M., Guengerich, F. P., & Shimada, T. (1998). Roles of cytochromes P450 1A2 and 3A4 in the oxidation of estradiol and estrone in human liver microsomes. *Chemical Research in Toxicology, 11*, 659–665.

86. Shou, M., Korzekwa, K. R., Brooks, E. N., Krausz, K. W., Gonzalez, F. J., & Gelboin, H. V. (1997). Role of human hepatic cytochrome P450 1A2 and 3A4 in the metabolic activation of estrone. *Carcinogenesis, 18*, 207–214.
87. Kawashiro, T., Yamashita, K., Zhao, X.-J., Koyama, E., Tani, M., Chiba, K., et al. (1998). A study on the metabolism of etoposide and possible interactions with antitumor or supporting agents by human liver microsomes. *Journal of Pharmacology and Experimental Therapeutics, 286*, 1294–1300.
88. Miyazawa, M., & Gyoubu, K. (2006). Metabolism of (+)-Fenchone by CYP2A6 and CYP2B6 in human liver microsomes. *Biological & Pharmaceutical Bulletin, 29*, 2354–2358.
89. Tateishi, T., Wood, A. J., Guengerich, F. P., & Wood, M. (1995). Biotransformation of tritiated fentanyl in human liver microsomes. Monitoring metabolism using phenyl acetic acid and 2-phenyl ethanol. *Biochemical Pharmacology, 50*, 1921–1924.
90. Guitton, J., Buronfosse, T., Desage, M., Lepape, A., Brazier, J. L., & Beaune, P. (1997). Possible involvement of multiple cytochrome P450s in fentanyl and sulfentanil metabolism as opposed to alfentanil. *Biochemical Pharmacology, 53*, 1613–1619.
91. Kleingeist, B., Bocker, R., & Geisslinger, G. (1998). Isolation and pharmacological characterization of microsomal human liver flumazenil carboxyl esterase. *Journal of Pharmacy & Pharmaceutical Sciences, 1*, 38–46.
92. Liu, Z. Q., Zhu, B., Tan, Y. F., Tan, Z. R., Wang, L. S., Huang, S. L., et al. (2002). *O*-dealkylation of fluoxetine in relation to CYP2C19 gene dose and involvement of CYP3A4 in human liver microsomes. *Journal of Pharmacology and Experimental Therapeutics, 300*, 105–111.
93. Tracy, T. S., Rosenbluth, B. W., Wrighton, S. A., Gonzalez, F. J., & Korzekwa, K. R. (1995). Role of cytochrome P450 2C9 and an allelic variant in the 4′-hydroxylation of (R)- and (S)- flurbiprofen. *Biochemical Pharmacology, 49*, 1269–1275.
94. Kang, P., Dalvie, D., Smith, E., Zhou, S., Deese, A., & Nieman, J. A. (2008). Bioactivation of flutamide metabolites by human liver microsomes. *Drug Metabolism and Disposition: The Biological Fate of Chemicals, 36*, 1425–1437.
95. Suzuki, A., Iida, I., Tanaka, F., Akimoto, M., Fukushima, K., Tani, M., et al. (1999). Identification of human cytochrome P450 isoforms involved in metabolism of R (+) - and S (−)-gallopamil: Utility of in vitro disappearance rate. *Drug Metabolism and Disposition: The Biological Fate of Chemicals, 27*, 1254–1259.
96. Bloomer, J. C., Baldwin, S. J., Smith, G. J., Ayrton, A. D., Clarke, S. E., & Chenery, R. J. (1994). Characterisation of the cytochrome P450 enzymes involved in the *in vitro* metabolism of granisetron. *British Journal of Clinical Pharmacology, 38*, 557–566.
97. Baune, B., Flinois, J. P., Furlan, V., Gimenez, F., Taburet, A. M., Becquemont, L., et al. (1999). Halofantrine metabolism in microsomes in man: Major role of CYP3A4 and CYP3A5. *Journal of Pharmacy and Pharmacology, 51*, 419–426.
98. Pan, L. P., Wijnant, P., De Vriendt, C., Rosseel, M. T., & Belpaire, F. M. (1997). Characterization of the cytochrome P450 isoenzymes involved in the in vitro *N*-dealkylation of haloperidol. *British Journal of Clinical Pharmacology, 44*, 557–564.
99. Hamman, M. A., Thompson, G. A., & Hall, S. D. (1997). Regioselective and stereoselective metabolism of ibuprofen by human cytochrome P450 2C. *Biochemical Pharmacology, 54*, 33–41.
100. Nakajima, M., Inoue, T., Shimada, N., Tokudome, S., Yamamoto, T., & Kuroiwa, Y. (1998). Cytochrome P450 2C9 catalyzes indomethacin *O*-demethylation in human liver microsomes. *Drug Metabolism and Disposition: The Biological Fate of Chemicals, 26*, 261–266.
101. Isoherranen, N., Kunze, K. L., Allen, K. E., Nelson, W. L., & Thummel, K. E. (2004). Role of itraconazole metabolites in CYP3A4 inhibition. *Drug Metabolism and Disposition: The Biological Fate of Chemicals, 32*, 1121–1131.
102. Liu, K. H., Kim, M. J., Jung, W. M., Kang, W., Cha, I. J., & Shin, J. G. (2005). Lansoprazole enantiomer activates human liver microsomal CYP2C9 catalytic activity in a stereo specific and substrate-specific manner. *Drug Metabolism and Disposition: The Biological Fate of Chemicals, 33*, 209–213.
103. Miyazawa, M., Shindo, M., & Shimada, T. (2002). Metabolism of (+)- and (−)-limonenes to respective carveols and perillyl alcohols by CYP2C9 and CYP2C19 in human liver microsomes. *Drug Metabolism and Disposition: The Biological Fate of Chemicals, 30*, 602–607.
104. Bonnabry, P., Leemann, T., & Dayer, P. (1996). Role of human liver microsomal CYP 2C9 in the biotransformation of lornoxicam. *European Journal of Clinical Pharmacology, 49*, 305–308.
105. Kobayashi, K., Mimura, N., Fujii, H., Minami, H., Sasaki, Y., Shimada, N., et al. (2000). Role of human cytochrome P450 3A4 in metabolism of medroxy progesterone acetate. *Clinical Cancer Research, 6*, 3297–3303.
106. Miyazawa, M., & Nakanishi, K. (2006). Biotransformation of (−)-menthone by human liver microsomes. *Bioscience, Biotechnoogy and Biochemistry, 70*, 1259–1261.

107. Foster, D. J. R., Somogyi, A. A., & Bochner, F. (1999). Methadone *N*-demethylation in human liver microsomes: Lack of stereo selectivity and involvement of CYP3A4. *British Journal of Clinical Pharmacology, 47*, 403–412.
108. Desta, Z., Wu, G. M., Morocho, A. M., & Flockhart, D. A. (2002). The gastro prokinetic and antiemetic drug metoclopramide is a substrate and inhibitor of cytochrome P450 2D6. *Drug Metabolism and Disposition: The Biological Fate of Chemicals, 30*, 336–343.
109. Koyama, E., Chiba, K., Tani, M., & Ishizaki, T. (1996). Identification of human cytochrome P450 isoforms involved in the stereo-selective metabolism of mianserin enantiomers. *Journal of Pharmacology and Experimental Therapeutics, 278*, 21–30.
110. Perloff, M. D., von Moltke, L. L., Court, M. H., Kotegawa, T., Shader, R. I., & Greenblatt, D. J. (2000). Midazolam and triazolam biotransformation in mouse and human liver microsomes: Relative contribution of CYP3A and CYP2C isoforms. *Journal of Pharmacology and Experimental Therapeutics, 292*, 618–628.
111. He, P., Court, M. H., Greenblatt, D. J., & von Moltke, L. L. (2006). Factors influencing midazolam hydroxylation activity in human liver microsomes. *Drug Metabolism and Disposition: The Biological Fate of Chemicals, 34*, 1198–1207.
112. Coleman, T., Ellis, S. W., Martin, I. J., Lennard, M. S., & Tucker, G. T. (1996). 1-Methyl-4-phenyl-1,2,3,6-tetra hydropyridine (MPTP) is *N*-demethylated by cytochromes P450 2D6, 1A2 and 3A4—Implications for susceptibility to Parkinson's disease. *Journal of Pharmacology and Experimental Therapeutics, 277*, 685–690.
113. Miners, J. O., Coulter, S., Tukey, R. H., Veronese, M. E., & Birkett, D. J. (1996). Cytochromes P450, 1A2 and 2C9 are responsible for the human hepatic *O*-demethylation of R- and S-naproxen. *Biochemical Pharmacology, 51*, 1003–1008.
114. Fujimaki, Y., Arai, N., Nakazawa, T., & Fujimaki, M. (2001). Nefiracetam metabolism by human liver microsomes: Role of cytochrome P450 3A4 and cytochrome P450 1A2 in 5-hydroxy nefiracetam formation. *Journal of Pharmacy and Pharmacology, 53*, 795–804.
115. Hecht, S. S., Hochalter, J. B., Villalta, P. W., & Murphy, S. E. (2000). 2′-Hydroxylation of nicotine by cytochrome P450 2A6 and human liver microsomes: Formation of a lung carcinogen precursor. *Proceedings of the National Academy of Sciences of the United States of America, 97*, 12493–12497.
116. Yoo, J.-S. H., Guengerich, F. P., & Yang, C. S. (1988). Metabolism of *N*-nitrosodialkylamines by human liver microsomes. *Cancer Research, 48*, 1499–1504.
117. Bellec, G., Dreano, Y., Lozach, P., Menez, J. F., & Berthou, F. (1996). Cytochrome P450 metabolic dealkylation of nine *N*-nitrosodialkylamines by human liver microsomes. *Carcinogenesis, 17*, 2029–2034.
118. Teiber, J. F., & Hollenberg, P. F. (2000). Identification of the human liver microsomal cytochrome P450s involved in the metabolism of *N*-nitrosodi-*n*-propyl amine. *Carcinogenesis, 21*, 1559–1566.
119. Olesen, O. V., & Linnet, K. (1997). Hydroxylation and demethylation of the tricyclic antidepressant nortriptyline by cDNA-expressed human cytochrome P-450 isozymes. *Drug Metabolism and Disposition: the Biological fate of Chemicals, 25*, 740–744.
120. Chiba, K., Kobayashi, K., Manabe, K., Tani, M., Kamataki, T., & Ishizaki, T. (1993). Oxidative metabolism of omeprazole in human liver microsomes: Cosegregation with S-mephenytoin 4′-hydroxylation. *Journal of Pharmacology and Experimental Therapeutics, 266*, 52–59.
121. Butler, A. M., & Murray, M. (1997). Biotransformation of parathion in human liver: Participation of CYP3A4 and its inactivation during microsomal parathion oxidation. *Journal of Pharmacology and Experimental Therapeutics, 280*, 966–973.
122. Bloomer, J. C., Woods, F. R., Haddock, R. E., Lennard, M. S., & Tucker, G. T. (1992). The role of cytochrome P450 2D6 in the metabolism of paroxetine by human liver microsomes. *British Journal of Clinical Pharmacology, 33*, 521–523.
123. Störmer, E., Brockmoller, J., Roots, I., & Schmider, J. (2000). Cytochrome P450 enzymes and FMO3 contribute to the disposition of the antipsychotic drug perazine in vitro. *Psychopharmacology, 151*, 312–320.
124. Davies, B. J., Herbert, M. K., Coller, J. K., Somogyi, A. A., Milne, R. W., & Sallustio, B. C. (2006). Determination of the 4-mono hydroxy metabolites of perhexiline in human plasma, urine and liver microsomes by liquid chromatography. *Journal of Chromatography B, Analytical Technologies in the Biomedical and Life Sciences, 843*, 302–309.
125. Olesen, O. V., & Linnet, K. (2000). Identification of the human cytochrome P450 isoforms mediating in vitro *N*-dealkylation of perphenazine. *British Journal of Clinical Pharmacology, 50*, 563–571.
126. von Moltke, L. L., Greenblatt, D. J., Duan, S. X., Schmider, J., Kudchadker, L., Fogelman, S. M., et al. (1996). Phenacetin *O*-deethylation by human liver microsomes in vitro: Inhibition by chemical probes, SSRI antidepressants, nefazodone and venlafaxine. *Psychopharmacology, 128*, 398–407.

127. Kudo, S., Umehara, K., Hosokawa, M., Miyamoto, G., Chiba, K., & Satoh, T. (2000). Phenacetin deacetylase activity in human liver microsomes: Distribution, kinetics and chemical inhibition and stimulation. *Journal of Pharmacology and Experimental Therapeutics, 294*, 80–88.
128. Desta, Z., Kerbusch, T., Soukhova, N., Richard, E., Ko, J. W., & Flockhart, D. A. (1998). Identification and characterization of human cytochrome P450 isoforms interacting with pimozide. *Journal of Pharmacology and Experimental Therapeutics, 285*, 428–437.
129. Wojcikowski, J., Pichard-Garcia, L., Maurel, P., & Daniel, W. A. (2003). Contribution of human cytochrome P450 isoforms to the metabolism of the simplest phenothiazine neuroleptic promazine. *British Journal of Clinical Pharmacology, 138*, 1465–1474.
130. Oda, Y., Hamaoka, N., Hiroi, T., Imaoka, S., Hase, I., Tanaka, K., et al. (2001). Involvement of human liver cytochrome P450 2B6 in the metabolism of propofol. *British Journal of Clinical Pharmacology, 51*, 281–285.
131. Shimizu, M., Matsumoto, Y., Tatsuno, M., & Fukuoka, M. (2003). Glucuronidation of propofol and its analogs by human and rat liver microsomes. *Biological and Pharmaceutical Bulletin, 26*, 216–219.
132. Johnson, J. A., Herring, V. L., Wolfe, M. S., & Relling, M. V. (2000). CYP1A2 and CYP2D6 4-hydroxylate propranolol and both reactions exhibit racial differences. *Journal of Pharmacology and Experimental Therapeutics, 294*, 1099–1105.
133. Zhao, X. J., Yokoyama, H., Chiba, K., Wanwimolruk, S., & Ishizaki, T. (1996). Identification of human cytochrome P450 isoforms involved in the 3-hydroxylation of quinine by human live microsomes and nine recombinant human cytochromes P450. *Journal of Pharmacology and Experimental Therapeutics, 279*, 1327–1334.
134. Wang, Y.-P., Yan, J., Fu, P. P., & Chou, M. W. (2005). Human liver microsomal reduction of pyrrolizidine alkaloid N-oxides to form the corresponding carcinogenic parent alkaloid. *Toxicology Letters, 155*, 411–420.
135. Xia, Q., Chou, M. W., Kadlubar, F. F., Chan, P.-C., & Fu, P. P. (2003). Human liver microsomal metabolism and DNA adduct formation of the tumorigenic pyrrolizidine alkaloid, Riddelline. *Chemical Research in Toxicology, 16*, 66–73.
136. Ueng, Y.-F., Don, M.-J., Jan, W.-C., Wang, S.-Y., Ho, L.-K., & Chen, C.-F. (2006). Oxidative metabolism of the alkaloid rutaecarpine by human cytochrome P450. *Drug Metabolism and Disposition: The Biological Fate of Chemicals, 34*, 821–827.
137. Baldwin, S. J., Clarke, S. E., & Chenery, R. J. (1999). Characterization of the cytochrome P450 enzymes involved in the *in vitro* metabolism of rosiglitazone. *British Journal of Clinical Pharmacology, 48*, 424–432.
138. Pedersen, R. S., Damkier, P., & Brosen, K. (2006). The effects of human CYP2C8 genotype and fluvoxamine on the pharmacokinetics of rosiglitazone in healthy subjects. *British Journal of Clinical Pharmacology, 62*, 682–689.
139. Manchee, G. R., Eddershaw, P. J., Ranshaw, L. E., Herriott, D., Park, G. R., Bayliss, M. K., et al. (1996). The aliphatic oxidation of salmeterol to alpha-hydroxy salmeterol in human liver microsomes is catalyzed by CYP3A. *Drug Metabolism and Disposition: The Biological Fate of Chemicals, 24*, 555–559.
140. Xu, Z. H., Wang, W., Zhao, X. J., Huang, S. L., Zhu, B., He, N., et al. (1999). Evidence for involvement of polymorphic CYP2C19 and 2C9 in the *N*-demethylation of sertraline in human liver microsomes. *British Journal of Clinical Pharmacology, 48*, 416–423.
141. Hyland, R., Roe, E. G., Jones, B. C., & Smith, D. A. (2001). Identification of the cytochrome P450 enzymes involved in the *N*-demethylation of sildenafil. *British Journal of Clinical Pharmacology, 51*, 239–248.
142. Taguchi, K., Konishi, T., Nishikawa, H., & Kitamura, S. (1999). Identification of human cytochrome P450 isoforms involved in the metabolism of S-2-[4-(3-methyl-2-thienyl) phenyl) propionic acid. *Xenobiotica, 29*, 899–907.
143. Crewe, H. K., Ellis, S. W., Lennard, M. S., & Tucker, G. T. (1997). Variable contribution of cytochromes P450 2D6, 2C9 and 3A4 to the 4-hydroxylation of tamoxifen by human liver microsomes. *Biochemical Pharmacology, 53*, 171–178.
144. Cresteil, T., Monsarrat, B., Alvineria, P., Treluyer, J. M., Vieira, I., & Wright, M. (1994). Taxol metabolism by human liver microsomes: Identification of cytochrome P450 isozymes involved in its biotransformation. *Cancer Research, 54*, 386–392.
145. Yun, C. H., Okerholm, R. A., & Guengerich, F. P. (1993). Oxidation of the antihistaminic drug terfenadine in human liver microsomes. Role of cytochrome P450 3A (4) in *N*-dealkylation and C-hydroxylation. *Drug Metabolism and Disposition: The Biological Fate of Chemicals, 21*, 403–409.
146. Volotinen, M., Turpeinen, M., Tolonen, A., Uusitalo, J., Maenpaa, J., & Pelkonen, O. (2007). Timolol metabolism in human liver microsomes is mediated principally by CYP2D6. *Drug Metabolism and Disposition: The Biological Fate of Chemicals, 35*, 1135–1141.

147. Purba, H. S., Back, D. J., & Orme, M. L. (1987). Tolbutamide 4-hydroxylase activity of human liver microsomes: Effect of inhibitors. *British Journal of Clinical Pharmacology, 24*, 230–234.
148. Wester, M. R., Lasker, J. M., Johnson, E. F., & Raucy, J. L. (2000). CYP2C19 participates in tolbutamide hydroxylation by human liver microsomes. *Drug Metabolism and Disposition, 28*, 354–359.
149. Srivastava, P. K., Yun, C. H., Beaune, P. H., Ged, C., & Guengerich, F. P. (1991). Separation of human liver microsomal tolbutamide hydroxylase and (*S*)-mephenytoin 4′-hydroxylase cytochrome P450 enzymes. *Molecular Pharmacology, 40*, 69–79.
150. Lasker, J. M., Wester, M. R., Aramsombatdee, E., & Raucy, J. L. (1998). Characterization of CYP2C19 and CYP2C9 from human liver: Respective roles in microsomal tolbutamide, *S*-mephenytoin and omeprazole hydroxylations. *Archives of Biochemistry and Biophysics, 353*, 16–28.
151. von Moltke, L. L., Greenblatt, D. J., Harmatz, J. S., Duan, S. X., Harrel, L. M., Cotreau-Bibbo, M. M., et al. (1996a). Triazolam biotransformation by human liver microsomes in vitro: Effects of metabolic inhibitors and clinical confirmation of a predicted interaction with ketoconazole. *Journal of Pharmacology and Experimental Therapeutics, 276*, 370–379.
152. Nakashima, A., Kawashita, H., Masuda, N., Saxer, C., Niina, M., Nagae, Y., et al. (2005). Identification of cytochrome P450 forms involved in the 4-hydroxylation of valsartan, a potent and specific angiotensin II receptor antagonist, in human liver microsomes. *Xenobiotica, 35*, 589–602.
153. Ball, S. E., Ahern, D., Scatina, J., & Kao, J. (1997). Venlafaxine: In vitro inhibition of CYP2D6 dependent imipramine and desipramine metabolism; comparative studies with selected SSRIs and effects on human hepatic CYP3A4, CYP2C9 and CYP1A2. *British Journal of Clinical Pharmacology, 43*, 619–626.
154. Fogelman, S. M., Schmider, J., Venkatakrishnan, K., von Moltke, L. L., Harmatz, J. S., Shader, R. I., et al. (1999). *O*- and *N*-demethylation of venlafaxine in vitro by human liver microsomes and by microsomes from cDNA-transfected cells: Effect of metabolic inhibitors and SSRI antidepressants. *Neuropsychopharmacology, 20*, 480–490.
155. Miyazawa, M., Sugie, A., & Shindo, M. (2002). Biotransformation of (−)-verbenone by human liver microsomes. *Bioscience, Biotechnology and Biochemistry, 66*, 2458–2460.
156. Miyazawa, M., Sugie, A., & Shimada, T. (2003). Roles of human CYP2A6 and 2B6 and rat CYP2C11 and 2B1 in the 10-hydroxylation of (−)-verbenone by liver microsomes. *Drug Metabolism and Disposition: The Biological Fate of Chemicals, 31*, 1049–1053.
157. Ghosal, A., Ramanathan, R., Yuan, Y., Hapangama, N., Chowdhury, S. K., Kishnani, N. S., et al. (2007). Identification of human liver cytochrome P450 enzymes involved in biotransformation of vicriviroc, a CCR5 antagonist. *Drug Metabolism and Disposition: The Biological Fate of Chemicals, 35*, 2186–2195.
158. Zhou, X. J., Zhou-Pan, X. R., Gauthier, T., Placidi, M., Maurel, P., & Rahmani, R. (1993). Human liver microsomal cytochrome P450 3A isozymes mediated vindesine biotransformation. *Biochemical Pharmacology, 45*, 853–861.
159. Zhou-Pan, X. R., Seree, E., Zhou, X. J., Placidi, M., Maurel, P., Barra, Y., et al. (1993). Involvement of human liver cytochrome P450 3A in vinblastine metabolism: Drug interactions. *Cancer Research, 53*, 5121–5126.
160. Zhao, X.-P., Zhong, J., Liu, X.-Q., & Wang, G.-J. (2007). CYP3A4 mediated *in vitro* metabolism of vinflunine in human liver microsomes. *Acta Pharmacologica Sinica, 28*, 118–124.
161. de Graeve, J., van Heugen, J. C., Zorza, G., Fahy, J., & Puozzo, C. (2008). Metabolism pathway of vinorelbine (Navelbine) in human: Characterization of the metabolites by HPLC–MS/MS. *Journal of Pharmaceutical and Biomedical Analysis, 47*, 47–58.
162. Kajita, J., Kuwabara, T., Kobayashi, H., & Kobayashi, S. (2000). CYP3A4 is mainly responsible for the metabolism of a new vinca alkaloid, vinorelbine, in human liver microsomes. *Drug Metabolism and Disposition: The Biological Fate of Chemicals, 28*, 1121–1127.
163. Renwick, A. B., Mistry, H., Ball, S. E., Walters, D. G., Kao, J., & Lake, B. G. (1998). Metabolism of zaleplon by human hepatic microsomal cytochrome P450 isoforms. *Xenobiotica, 28*, 337–348.
164. Renwick, A. B., Ball, S. E., Tredger, J. M., Price, R. J., Walters, D. G., Kao, J., et al. (2002). Inhibition of zaleplon metabolism by cimetidine in the human liver: In vitro studies with subcellular fractions and precision-cut liver slices. *Xenobiotica, 32*, 849–862.
165. Fayz, S., & Inaba, T. (1998). Zidovudine azido-reductase in human liver microsomes: Activation by ethacrynic acid, dipyridamole and indomethacin and inhibition by human immunodeficiency virus protease inhibitors. *Antimicrobial Agents and Chemotherapy, 42*, 1654–1658.
166. Shiraga, T., Kaneko, H., Iwasaki, K., Tozuka, Z., Suzuki, A., & Hata, T. (1999). Identification of cytochrome P450 enzymes involved in the metabolism of zotepine, an antipsychotic drug, in human liver microsomes. *Xenobiotica, 29*, 217–229.
167. Lowry, O. H., Rosebrough, N. J., Farr, A. L., & Randall, R. J. (1951). Protein measurement with the folin phenol reagent. *Journal of Biological Chemistry, 193*, 265–275.

168. Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anaytical Biochemistry, 72*, 248–254.
169. Omura, T., & Sato, R. (1964). The carbonmonoxide binding pigment of liver microsomes. *Journal of Biological Chemistry, 239*, 2370–2378.
170. Murai, T., Iwabuchi, H., & Ikeda, T. (2004). Identification of gemfibrozil metabolites, produced as positional isomers in human liver microsomes, by on line analyses using liquid chromatography/mass spectrometry and liquid chromatography/nuclear magnetic resonance spectroscopy. *Journal of the Mass Spectrometry Society of Japan, 52*, 277–283.
171. Segel, I. H. (1993). *Enzyme kinetics: Behavior and analysis of rapid equilibrium and steady-state enzyme systems*. New York: Wiley.
172. Chiba, K., Saitoh, A., Koyama, E., Tani, M., Hayashi, M., & Ishizaki, T. (1994). The role of *S*-mephenytoin 4′-hydroxylase in imipramine metabolism by human liver microsomes: A two enzyme kinetic analysis of *N*-demethylation and 2-hydroxylatlion. *British Journal of Clinical Pharmacology, 37*, 237–242.
173. Hargreaves, M. B., Jones, B. C., Smith, D. A., & Gescher, A. (1994). Inhibition of *p*-nitrophenol hydroxylase in rat liver microsomes by small aromatic and heterocyclic molecules. *Drug Metabolism and Disposition: The Biological Fate of Chemicals, 22*, 806–810.
174. Obach, R. S. (1997). Nonspecific binding to microsomes: Impact on scale-up of in vitro intrinsic clearance to hepatic clearance as assessed through examination of warfarin, imipramine and propranolol. *Drug Metabolism and Disposition: The Biological Fate of Chemicals, 25*, 1359–1369.
175. Stresser, D. M., & Kupfer, D. (1999). Monospecific antipeptide antibody to cytochrome P-450 2B6. *Drug Metabolism and Disposition: The Biological Fate of Chemicals, 27*, 517–525.
176. Beaune, P. H., Kremers, P. G., Kaminsky, L. S., de Graeve, J., Albert, A., & Guengerich, F. P. (1986). Comparison of monooxygenase activities and cytochrome P450 isozyme concentration in human liver microsomes. *Drug Metabolism and Disposition: The Biological Fate of Chemicals, 14*, 437–442.
177. Guengerich, F. P., & Shimada, T. (1991). Oxidation of toxic and carcinogenic chemicals by human cytochrome P450 enzymes. *Chemical Research in Toxicology, 4*, 391–407.
178. Zuber, R., Modriansky, M., Dvorak, Z., Rohovsky, P., Ulrichova, J., Simanek, V., et al. (2002). Effect of silybin and its congeners on human liver microsomal cytochrome P450 activities. *Phytotherapy Research, 16*, 632–638.