Minireview
Antibodies as a Probe in Cytochrome P450 Research
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ABSTRACT:
Cytochrome P450 (P450) is the superfamily of enzymes responsible for biotransformation of endobiotics and xenobiotics. However, their large isomorph multiplicity, inducibility, diverse structure, widespread distribution, polymorphic expression, and broad overlapping substrate specificity make it difficult to measure the precise role of each individual P450 to the metabolism of drugs (or carcinogens) and hamper the understanding of the relationship between the genetic/environmental factors that regulate P450 phenotype and the responses of the individual P450s to drugs. The antibodies against P450s have been useful tools for the quantitative determination of expression level and contribution of the epitope-specific P450 to the metabolism of a drug or carcinogen substrate in tissues containing multiple P450 isoforms and for implications in pharmacogenetics and human risk assessment. In particular, the inhibitory antibodies are uniquely suited for reaction phenotyping that helps to predict human pharmacokinetics for clinical drug-drug interaction potential in drug discovery and development.

Many enzyme systems exist in the liver and in extrahepatic tissues, such as intestine and kidney, that can contribute to the clearance of drugs from the systemic circulation. Among the various phase I and phase II drug-metabolizing enzymes, the cytochrome P450 (P450) enzymes play a key role in xenobiotic and endobiotic metabolic processing. It has been estimated that approximately two thirds of marketed drugs are metabolized by this enzyme system (Rendic and Di Carlo, 1997; Guengerich, 2003; Williams et al., 2004). In fact, members of three P450 subfamilies (CYP1, CYP2, and CYP3) are largely responsible for the metabolic clearance of drugs and xenobiotics (Rendic and Di Carlo, 1997; Guengerich, 2003; Williams et al., 2004). Multiplicity and overlapping substrate specificity of P450 have greatly complicated efforts at understanding the precise role of individual P450 isoforms in the metabolism of drugs and drug candidates. Therefore, over recent years, a variety of reagents and tools have been developed for in vitro and in vivo studies, and it is now possible to determine which specific P450 isoform(s) is (are) involved in the metabolism of a given compound (Rodrigues, 1999; Rodrigues and Rushmore, 2002; Lu et al., 2003; Williams et al., 2003). Given the importance of fraction of drug metabolized (f_m) in assessing the potential of in vivo drug-drug interaction (DDI), P450 reaction-phenotyping studies are conducted at multiple stages during drug discovery and development. Irrespective of the strategy, information related to P450-mediated metabolism is included in regulatory documents and the absorption, distribution, metabolism, and elimination (ADME) and DDI sections of the product label, where precautions concerning the coadministration of other drugs are listed along with recommendations for dose adjustment (Obach et al., 2006; Huang et al., 2007). Antibodies that are specific to individual P450s can be used to determine P450 protein content by immunoreactivity and quantify the role of the target P450 in tissue preparations (i.e., liver microsomes) by their inhibition of substrate-specific metabolism. Therefore, quantitative knowledge of the P450-specific metabolism of a drug and the individual’s phenotype by the antibodies in drug discovery and development are important in the prediction of efficacy, dosage, and toxicity of the drug candidate, individual variability of patients in response to the drug, and potential DDI. In this article, we will briefly overview types and generation of P450-directed antibodies and their applications in P450 research, drug development, and human risk assessment.

Antibodies against P450s

Polyclonal Antibodies. Polyclonal antibodies isolated from rabbits immunized against purified hepatic P450s from either 3-methylcholanthrene (3MC)- or phenobarbital (PB)-treated rats were first developed by Thomas et al. (1976a,b). Using these antibodies, six different isoforms of rat liver P450s were detected by Ouchterlony immunodiffusion. In addition, the antibodies raised from 3MC- and PB-treated rats exhibited inhibitory effects on purified rat CYP1A (MC-448)-mediated benzo[a]pyrene hydroxylation and CYP2B (PB-P450)-mediated benzphetamine N-demethylation reactions, respectively. The antibody inhibition patterns of the various substrates were also observed in

ABBREVIATIONS: P450, cytochrome P450; f_m, fraction of drug metabolized; DDI, drug-drug interaction; ADME, absorption, distribution, metabolism, and elimination; 3MC, 3-methylcholanthrene; PB, phenobarbital; PCN, pregnenolone-16a-carbonitrile; MAb, monoclonal antibody; ELISA, enzyme-linked immunosorbent assay; RIA, radioimmunoassay; HLM, human liver microsomes; GST, glutathione S-transferase; PMNs, polymorphic neutrophils; AFB_1, aflatoxin B_1; NHBE, normal human bronchial epithelial.
native liver microsomes of rats treated with 3MC, PB, and pregnenolone-16α-carbonitrile (PCN), respectively (Thomas et al., 1977). These antibody inhibition patterns of marker substrate metabolism helped identify one or multiple P450 isoforms in rat liver microsomes.

**Monoclonal Antibodies.** The hybridoma technology (Kohler and Milstein, 1975) used to generate monoclonal antibodies (MAbs) against individual P450s was a major achievement in antibody development. With this technology, each B-lymphocyte antibody-forming cell and its progeny is committed to the production of a single, unique type of antibody molecule (Yelton and Scharff, 1981). The procedures include the following: 1) immunization of mice by injection of pure P450 antigens; 2) hybridoma production by fusion of spleen and myeloma cells; 3) cloning of the hybridomas to a monoclonal hybridoma cell that produces antibodies directed to a single epitope; 4) screening for the produced antibodies using enzyme-linked immunosorbent assay (ELISA) and inhibition of marker P450 activity; and 5) production of the antibodies by cell culture of the hybridomas or in ascites fluid of mice receiving intraperitoneal injections of the hybridomas (Gelboin et al., 1998). The generated MAb molecule specifically recognizes and binds a single “epitope” on the P450 protein antigen. The hybridoma cell line serves as an immortal source of producing the limitless epitope-specific antibody proteins. MAbs have been widely used in the study of multiple P450 isoforms and are far superior to polyclonal antibodies, which are likely to exhibit considerable undesired cross-reactivity.

Production and characterization of MAbs against individual P450s (CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1, CYP3A4, etc.) has been published by Gelboin’s laboratory (Gelboin, 1993; Gelboin et al., 1995, 1996, 1997, 1998, 1999; Yang et al., 1998a,b; Sai et al., 1999; Krausz et al., 2001). These antibodies are specific to the P450s with immunoblot and inhibitory properties, which can be used to assess expression level of the individual P450s in tissues in response to the treatment of an inducer and to quantitatively measure contribution of the P450s to the metabolism of a drug or chemical including toxins, mutagens, and carcinogens (Gelboin, 1993; Gelboin et al., 1998, 1999; Yung et al., 1998c, 1999; Meit et al., 1999; Shou et al., 2000). Although MAbs have some advantages in P450 identification and quantification studies, there are a number of concerns in their application of MAbs. First, a library of inhibitory MAbs for all human individual P450 isoforms is not commercially available. Second, some MAbs may not achieve complete inhibition. The reason for this could be that MAbs-enzyme-substrate complex is still productive, leading to a partial inhibition (Gelboin et al., 1995). Third, some current MAbs cannot distinguish between closely related P450 subfamily members (i.e., CYP3A4 and CYP3A5) (Gelboin, 1993).

**Peptide-Directed P450 Antibodies.** Antibodies against P450s can be produced by immunizing animals with synthetic peptides that mimic small regions of the apoprotein. Because the amino acid sequences of a number of P450s are known, this approach to generate antibodies can be applied to most of the major P450s in human and other species. This is a relatively simple, rapid, and effective method of producing antibodies. The anti-peptide approach to antibody production overcomes the need to use purified P450s as immunogens. A high degree of binding specificity can be achieved by directing anti-peptide antibodies toward unique regions of the target antigen (Edwards et al., 1993, 1995). These antibodies are particularly suited for immunoblotting, immunocytochemistry, and immunoinhibition of enzyme activity (Edwards et al., 1990, 1991; Wang et al., 1999).

Unlike antibodies produced by other techniques, the epitopes of these antibodies are predetermined, allowing them to be directed toward specific regions of P450 isoforms. Detailed practical information was described by Edwards (2006) on the selection of regions to target, the synthesis and conjugation of peptides to carrier protein, immunization, and the assessment of the resultant antisera. Peptide-directed antibodies against human CYP1A1, CYP1A2, CYP1B1, CYP2A6, CYP2B6, CYP2D6, CYP2E1, CYP3A4, CYP3A5, and CYP4A11 and an antibody that binds to CYP2C8, CYP2C9, and CYP2C19 have been produced by immunoraising rabbits with synthetic peptides representing small regions of each of these P450 enzymes. The specificity of the antibodies was confirmed by immunoblotting using recombinant P450 enzymes and samples of human hepatic microsomal membranes. The results demonstrated the utility of the anti-peptide approach for producing specific antibodies against human P450 enzymes, enabling a comprehensive panel of antibodies against human P450s to be produced (Edwards et al., 1998). The major drawback of this approach was that most of the anti-peptide antibodies were noninhibitory. Even when inhibitory antibodies can be produced in some rabbits, consistent productions of inhibitory antibodies in all rabbits remain a technical challenge (Wang et al., 1999).

**Application**

**Probing the Membrane Topology of P450.** The liver microsomal P450s were first characterized in the early 1960s (Omura and Sato, 1964). One of the areas of special interest to investigators at that time was the spatial arrangement and localization of both P450 enzyme and NADPH-P450 reductase in the microsomal membrane because this is important for the understanding of the interaction of these two components and the mechanism of microsomal electron transport. The hydrophilic portion of the reductase can be cleaved and released from the microsomal membrane by protease treatment, whereas the hydrophobic tail of the reductase is embedded in the lipid bilayer of the microsomal membrane. The bulk of the protein containing the active site of the enzyme was exposed to the cytosolic side of the lipid bilayer based on protease digestion and inhibition of metabolism by antibodies (Black and Coon, 1982). In contrast, either P450 or its denatured form, P420, was not solubilized by protease treatment, raising the possibility that P450 may be deeply embedded in the microsomal membrane.

Antibodies produced against P450s play a prominent role in the investigation of membrane topology of P450. Initially, inhibition of liver microsomal metabolism of drugs and other substrates was used to probe the accessibility of P450 in the microsomal membrane (Thomas et al., 1977). Strong antibody inhibition of the microsomal metabolism indicated that P450 was at least partially exposed to the hydrophilic environment on the exterior of liver microsomal membrane. Subsequently, antibodies against the N-terminal amino acid sequences were used to examine whether they can bind to the specific region of P450 in the microsomal membrane. Using antibodies prepared against peptides in various lengths from the N terminus to residue 38 of CYP2B1, De Lemos-Chiarandini et al. (1987) found that an antibody raised to the first 31 residues of CYP2B1 bound well to the purified enzymes but poorly to the microsomal preparations. In contrast, an antibody prepared against residues 24 to 38 showed strong binding to intact liver microsomes. These results strongly suggested that approximately the first 20 amino acids from the amino terminal of CYP2B1 are embedded in the phospholipid bilayer and the rest of the enzyme is exposed to the cytoplasmic surface of the membrane.

Based on proteolysis study, chemical modification, and theoretical considerations, two topological models were proposed for P450 (Black, 1992). One model proposes a single trans-membrane anchor peptide (approximately the first 20 amino acids) with the amino terminus of P450 projected into the lumen of the endoplasmic reticulum. The other model suggested a hairpin loop of the first approx-
imately 46 residues inserted into the membrane with the amino terminus located on the cytosolic side of the lipid bilayer. To distinguish between these two possibilities, Black et al. (1994) used monoclonal antibodies prepared against residues 18 to 29 of CYP2B4, corresponding to the heme-transfer signal of the enzyme. Based on these two models, the cationic heme-transfer signal peptide of CYP2B4 (residues 21–29) should be located at the cytosolic surface of the membrane in the first model but at the luminal side of the membrane in the second model. ELISAs showed that all of the antibodies exhibited strong binding to intact liver microsomes. No enhanced binding by any of the antibodies could be observed in detergent-solubilized microsomes. Thus, the heme-transfer signal of CYP2B4 was localized at the cytosolic side of the microsomal membrane, and no fraction of the peptide segment was found in the lumen, consistent with the model that a single trans-membrane peptide at the N terminus was embedded in the microsomal membrane, while the rest of CYP2B4 was exposed to the cytosolic side of the lipid bilayer. This P450 membrane topology model has been widely accepted (Johnson, 2003).

Antibodies are extraordinarily suitable for investigation of the molecular and functional diversity of P450s. The P450s are present in multiple forms, and the levels of each isoform vary largely in response to drugs, endogenous substances, and physiological and environmental states. Therefore, antibodies can be used for identification and quantification of the P450 proteins under the different conditions. Detection of epitope-specific P450s in organs, tissues, cells, and subcellular organelles can be accomplished with immunoassays, such as radioimmunoassay (RIA), ELISA, and immunoblotting. RIA assays are rapid and sensitive and can detect P450 at a level of <100 ng of microsomal protein (Cheng et al., 1984; Song et al., 1985). The MAb-based immunochemistry can be applied to localize and examine the distribution of individual cytochrome P450s after different inducer administration. The first application to the analysis of CYP1A1/2 in liver cells showed detection of the epitope containing P450s in 3MC-induced rat liver and is localized in both the rough and smooth endoplasmic reticulums as well as the nuclear envelope of hepatocytes (Brands et al., 1985). The method provided a variety of utility to examine the cellular distribution of P450s in the liver and the extrahepatic tissues (Foster et al., 1986).

Probing the Multiplicity of P450. Enzyme multiplicity was one of the most dominant issues in P450 research in the 1970s. Before recombinant DNA technology was available, classic biochemical approaches involving multiple purification steps were used to purify either a single P450 isoform or a mixture of structurally related P450 enzymes. Antibodies against these purified enzymes played a prominent role in the demonstration that multiple species of P450 are involved in the metabolism of drugs and other substrates.

Interaction studies between purified P450s from PB- as well as 3MC-treated rats with their respective antibodies illustrate the value of this approach before all individual rat P450s were purified and characterized. Even though P450s purified from these inducer-treated rats were electrophoretically pure, as judged by a single staining protein band in sodium dodecyl sulfate-gel electrophoresis under the assay condition, confirmation of homogeneity of these enzyme preparations was required by a different experimental approach. Using the classic Ouchterlony double-diffusion technique and antibodies prepared against the purified P450s from PB- and 3MC-treated rats, Thomas et al. (1976a) found four antigenically different forms of P450s in the purified P450 preparation from PB-treated rats and two different forms in the purified preparation from 3MC-treated rats. These results were later confirmed by further purification studies and the cloning of cDNA encoding various P450s in rats. A similar approach has also been used to demonstrate structural relatedness among various P450s isolated from the same animal (Reik et al., 1982; Bandiera et al., 1985).

Probing Human P450s. From the early 1970s to mid 1980s, purification and characterization of P450s were mostly carried out in animal species. When human liver samples became widely available in the mid 1980s, investigators, led by F. P. Guengerich and P. S. Guzelian, started to isolate and characterize human P450s. Antibodies again played a significant role in this investigation. Using a different approach, Guengerich and colleagues (Guengerich et al., 1986; Butler et al., 1989) purified a number of P450 isoforms from human liver microsomes (HLM) by following the catalytic activities of the metabolism of specific substrates such as nifedipine. Again, antibodies prepared against various P450s purified from rat and human livers were valuable tools to characterize the purified P450 isoforms from human liver microsomes, and they laid the foundation of our current knowledge of human P450.

Initially, PCN was found to induce a P450 isoform in rats different from the PB- or 3MC-inducible P450s based on spectral and catalytic properties (Lu et al., 1972). In 1980, Elshourbagy and Guzelian (Elshourbagy and Guzelian, 1980) isolated a P450 species, named P450p, from liver microsomes of PCN-treated rats. Although this P450 species exhibited little catalytic activity toward several substrates when reconstituted with NADPH-P450 reductase and phospholipid, a potent antibody was produced against this P450. Antibody against P450p strongly inhibited the metabolism of several substrates not only from liver microsomes of PCN- or triacetyletoleandomycin-treated rats, but also from liver microsomes of human subjects (Watkins et al., 1985; Wrighton et al., 1985). This antibody cross-reacted strongly with CYP3A4, the major P450 isoform in human liver, and was a valuable tool for CYP3A4 research for many years.

Human Risk Assessment. The association of P450 genetic polymorphism and human cancer risk, and susceptibility to environmental hazards, has received increasing attention. In addition, the current status and perspectives of using P450 genetic polymorphism as a biomarker of individual susceptibility to cancer and environmental toxicity are research areas of great interest (Hong and Yang, 1997). The expression of P450 genes in target cells is an important determinant of human susceptibility to cancers and other chemically initiated diseases. In these studies, antibodies directed against various human P450s are valuable tools for the localization and characterization of P450s in all kinds of human tissues. For example, use of immunohistochemistry allowed investigation on the cellular distribution and localization of CYP1A1 and CYP1B1 in human skin and their induction by UV B. Human CYP1A1 was found to be primarily localized in the basal cell layer of the epidermis in non-UV B-exposed skin, whereas CYP1B1 was localized in the epidermal cells other than the basal cell layer. Thus, localizations of CYP1A1 and CYP1B1 in human skin are different and may be related to keratinocyte differentiation. UV B induction of both enzymes in human skin will probably result in enhanced bioactivation of polycyclic aromatic hydrocarbons and other environmental pollutants to which humans are exposed, which in turn could make the human skin more susceptible to UV B-induced skin cancers or allergic and irritant contact dermatitis (Katiyar et al., 2000).

Chronic pancreatitis and pancreatic cancer have been linked to the exposure of environmental chemicals (xenobiotics), which generally require metabolic activation to highly reactive toxic or carcinogenic intermediates. The primary enzyme system involved is made up of numerous P450s. A study was performed with immunochemical detection of CYP1A1, 1A2, 2B6, 2C8/9/19, 2D6, 2E1, 3A4, glutathione S-transferase (GST)-α, GST-μ, and GST-π, and NADPH P450 oxidoreductase in normal pancreatic tissue specimens (Standop et al., 2018).
2002). In all pancreatic regions, most of the enzymes were expressed in islet cells. However, more islets in the head region expressed CYP2B6, 2C8/9/19, 2E1, and oxidoreductase than those in the body and tail. Moreover, the expression of P450 2B6 and 2E1 was restricted to the pancreatic polypeptide (PP) cells, and the concentration of CYP3A4 was higher in PP cells than in other islet cells. The greater content of xenobiotic-metabolizing and carcinogen-activating P450 enzymes and a lower expression of detoxifying GST enzymes in the head of the pancreas could be one reason for the greater susceptibility of this region for inflammatory and malignant diseases.

Anti-CYP1B1 has been used for the immunohistochemical localization of CYP1B1 in breast cancer (McFadyen et al., 1999). The lung represents an important target for the toxic effects of chemicals. Using either individual human lung samples or pooled microsomes from different individuals, immunoreactive bands specific for the following P450 enzymes were determined by immunoblotting: CYP1A1, CYP1A2, CYP1B1, CYP2C, CYP2A, CYP2B6, CYP2D6, CYP2E1, and CYP3A4/5 (Bernauer et al., 2006). Study on expression and induction of CYP3A4 in different peripheral blood cell populations was performed. Rifampicin (600 mg/day for 6 days) was given to 8 healthy subjects. Human polymorphic neutrophils (PMNs) and lymphocytes were isolated by centrifugation of whole blood cell fractions. PMN and lymphocyte smears and homogenates were subjected to immunostaining and immunoblotting, respectively, with a mouse monoclonal antibody recognizing all CYP3A proteins. These results demonstrate that CYP3A proteins are constitutively expressed not only in PMNs but also in lymphocytes. However, in both cell lineages, CYP3A protein expression was not induced by rifampicin (Starkel et al., 1999).

The expression of P450s in human esophageal mucosa was investigated in a total of 25 histologically non-neoplastic surgical tissue specimens by using specific antibodies in immunoblots analysis. The presence of CYP1A, 2E1, 3A, and 4A enzymes was demonstrated by both techniques; CYP2A-reactive protein was also detected by immunoblot. These data demonstrate that a range of P450 enzymes are expressed in human esophageal mucosa and indicate that this tissue has the capacity to activate chemical carcinogens to reactive DNA binding metabolites (Lechevrel et al., 1999).

Aflatoxin B$_1$ (AFB$_1$) is a potent hepatocarcinogen and a risk factor for human lung cancer in certain occupations. To study the potential for AFB activation in human lung, P450-mediated activation and GST-mediated detoxification of AFB$_1$ were examined in cultured normal human bronchial epithelial (NHBE) cells. Western immunoblots showed that the primary P450 isoforms responsible for AFB activation in the liver, 1A, and 3A4, were constitutively expressed in NHBE cells. Expression of CYP1A1 was significantly increased in 3MC-pretreated cells, whereas CYP3A4 expression increased slightly, but not to the extent of the 1A isoforms. The principal AFB oxide-detoxifying enzyme, GST, was constitutively expressed in NHBE cells and was increased approximatley two-fold by 3MC pretreatment. These data support modest P450-mediated AFB$_1$ activation in human airways and indicate that exposure to polycyclic aromatic hydrocarbons, such as 3MC, which induce P450(s) that specifically activate AFB$_1$, may increase the harmful effects of AFB$_1$ exposures in human airways (Van Vleet et al., 2001). In addition to xenobiotic-metabolizing P450s, monoclonal antibodies to human aromatase (CYP19) have also been isolated and characterized (Turner et al., 2002). Human aromatase is believed to be a potential therapeutic target for the treatment of certain breast cancers.

**Reaction Phenotyping of Individual P450s.** Drug metabolism in humans is catalyzed by more than 10 microsomal P450s that are heterogeneously distributed in the liver and other tissues. Five of them (CYP1A2, CYP2C9, CYP2C19, CYP2D6, and CYP3A4) are involved in ~95% of the P450-mediated metabolism of drugs (approximately 75% of all drug substrates) (Williams et al., 2004). Because each microsomal P450 can metabolize multiple drugs and produce multiple metabolites (Rendic and Di Carlo, 1997; Guengerich, 2003), a general principle in drug development is to avoid the development of new drugs that can cause potent inhibition and induction of drug-metabolizing P450s. To this end, identification of P450s responsible for the metabolism of new therapeutic agents is pivotal in drug development. Multiple approaches have been used for P450 identification (Lu et al., 2003), which include uses of cDNA-expressed P450s in various systems, chemical inhibitors selective and antibodies specific to the individual P450s etc. Potent, specific, and inhibitory antibodies against various human P450s represent one of the most valuable approaches for P450 identification (Gelboin, 1993; Gelboin et al., 1999; Mei et al., 1999; Shou et al., 2000; Lu et al., 2003; Gelboin and Krausz, 2006). If the inhibition potency and isofrom specificity of a particular antibody is verified, the antibody can be chosen as the ideal approach necessary for a P450 phenotyping study. Thus, within a mixture of P450s, as is present in common tissue preparations such as microsomes or cell homogenates, the inhibitory antibodies can measure the contribution of a single P450 to the metabolism of a specific substrate. The amount of conversion of a substrate to a product catalyzed by a specific P450 can be quantitatively measured by the amount of inhibition of activity caused by the inhibitory antibody (Nguy et al., 2000; Tang et al., 2000; Kassahun et al., 2001). Reaction phenotyping is important with respect to the toxicity of a drug, DDIs, and a drug administered to an individual that is polymorphically defective in certain P450 isoforms, such as CYP2D6 (Lin and Lu, 1998, 2001). Antibodies that are specific to individual P450s can be used to determine P450 protein content by immunoblot and to quantify the role of the target P450 in tissue preparations (liver microsomes) by their inhibition of substrate metabolism (Gelboin, 1993; Gelboin et al., 1995; Yang et al., 1999; Shou et al., 2000; Gelboin and Krausz, 2006). Drugs primarily metabolized by a single P450 can be used as markers to identify the presence and the activity of the target P450 in vivo. In addition, antibodies can be used to study polymorphic P450s and to examine the metabolic consequences of an absent or deficient polymorphic P450 in individual liver microsomes (Gelboin et al., 1999). If a drug is metabolized by multiple P450s in HLM, the partial inhibition represents the contribution of the inhibited enzyme to the metabolism of the drug. Combined use of multiple antibodies examines the role of the target P450 in the presence of other P450s competing for the same drug substrate (Gelboin and Krausz, 2006).

To conduct a reaction phenotyping study with inhibitory antibodies, saturating concentrations of antibodies in the incubation containing multiple P450s (i.e., HLM) are necessary to measure the maximum contribution of each P450 for the metabolism of the drug. Thus, to achieve the saturating level of antibody, a titration curve needs to be performed to define the maximum inhibition in HLM at known microsomal protein concentrations. Kinetic nature of antibody inhibition in P450-mediated reactions has been characterized to show a noncompetitive kinetics, which causes an increase in $K_m$ but a decrease in $V_{max}$ as the antibody concentration increases (Mei et al., 2002).

**Clinical Implications.** Identification of a P450 responsible for the metabolism of a drug is important with respect to clinical DDIs, genetic polymorphism, and toxicity (toxicant formation, mutagenesis, carcinogenesis, DNA and protein adduct formation, and immunotoxicity). There are many examples in which drug metabolism is implicated in the toxicity of therapeutic agents either through impaired clearance in the case of polymorphic mechanisms or through in-
creased exposure in the case of reactive or toxic metabolites (Nakamura et al., 1985; Hong and Yang, 1997; Bartsch et al., 2000; Song et al., 2001; Ingelman-Sundberg, 2002; Knowles et al., 2003; Agundez, 2004; Sanderson et al., 2005). Impairment of drug metabolism due to enzyme inhibition has obvious clinical implications including toxicity as a result of increased bioavailability and decreased clearance. Reaction phenotyping can help understand the potential mechanism of toxicity and identify individual susceptibility to such toxicity. For instance, drugs metabolized primarily by CYP2D6, CYP2C19, or CYP2C9 are likely to be subject to significant interindividual variability in their exposure and effects as a result of known genetic polymorphisms. Fluoxetine (CYP2D6), omeprazole (CYP2C19), and warfarin (CYP2C9) are metabolized by the polymorphic enzymes and are still used in clinic; however, caution should be taken for the safety margins with patients with known genotype/phenotype (e.g., the CYP2C19 slow metabolizer phenotype is much more prevalent in Asians than in whites) (Goldstein et al., 1997; Shimoda et al., 1999; Yokono et al., 2001; Mizutani, 2003; Niu et al., 2004; Bertilsson, 2007).

Evaluation of the significance of the P450 reaction-phenotyping data requires the integration of information obtained from various in vitro systems, as well as integration of clinical data (e.g., human radiolabel study and drug interaction studies with probe drugs), allowing one to estimate the fraction of the dose cleared via all P450s ($f_m$), and the contribution of each P450 to total P450-dependent clearance ($f_{m,CYP}$). Integration of the data is key because it is the product of $f_m$ and $f_{m,CYP}$ (i.e., $f_m f_{m,CYP}$) that governs the magnitude of a drug interaction, and the impact of a P450 polymorphism on the pharmacokinetic profile of the victim drug in question. As such information becomes available, more effective clinical studies can be designed and conducted to avoid therapeutic failure (due to the induction of metabolic clearance) and unexpected toxicity (due to overdose resulting from impaired metabolic clearance via polymorphic enzymes or enzyme inhibition). Likewise, in vitro metabolism studies can help medicinal chemists to modify structure of molecules that could minimize or eliminate “metabolic liabilities” during the discovery stage (Rodrigues, 1999).

Several polymorphisms in the P450 enzyme have been reported as a result of single nucleotide polymorphisms, gene deletions, and gene duplications (Rodrigues and Rushmore, 2002). CYP2D6 and CYP2C19 are polymorphically expressed in different populations such as Asian and white (Nakamura et al., 1985; Kimura et al., 1998). If the in vivo clearance of a drug is largely mediated by a polymorphically expressed allelic variant of a P450, it is anticipated that poor metabolizers will produce elevated plasma area under the curve and/or an increased half-life, adversely affecting the safety and efficacy of drugs. In addition, the catalytic activity of CYP3A4-dependent midazolam hydroxylation has been reported to range from 95 to 651 pmol min$^{-1}$mg$^{-1}$ among pooled HLMs from 20 donors (Huang et al., 2004). This wide range of the activity might cause interindividual variability for drug exposure. Therefore, it is important to determine the P450 contribution to the metabolism of a drug considering the attrition of drug candidates in the discovery process. A growing number of drugs have labels with recommended dose adjustments based on reaction phenotyping and/or genotype information pertaining to drug-metabolizing enzymes (Sanderson et al., 2005; Nagar and Blanchard, 2006).

With the advent of polypharmacy, a competitive marketplace, and greater attention paid to public safety (i.e., risk versus benefit), pharmaceutical companies have increasingly focused on new chemical entities with optimal pharmacokinetic/ADME properties that preclude costly therapeutic monitoring and genotyping of patients (Lin and Lu, 1997; Rodrigues and Rushmore, 2002). It is also advantageous to develop new chemical entities with minimal DDI liabilities, so that package insert (“black box”) warnings can be avoided and patient safety maximized as much as possible. The highly publicized market withdrawals of two DDI “victim” drugs, terfenadine and cerivastatin, were precipitated partially because of a drug interaction involving the inhibition of CYP3A4 and CYP2C8, respectively (Honig et al., 1993; Backman et al., 1994; Varhe et al., 1994; Gomez et al., 1995; Floren et al., 1997; Azie et al., 1998; Gorski et al., 1998; Greenblatt et al., 1998; Backman et al., 2002; Emoto et al., 2006). More recently, drug interactions involving the inhibition of tizanidine (CYP1A2-dependent), repaglinide (CYP2C8/CYP3A4-dependent), and fluticasone (CYP3A4-dependent) clearance have also received some attention (Niemi et al., 2003; Arrington-Sanders et al., 2006; Backman et al., 2006). In addition, there are examples of successfully marketed “back up” drugs that have improved DDI profiles and less dependence on polymorphic P450s for clearance (e.g., fexofenadine versus terfenadine).

In general, the consequences of higher metabolic clearance are tied to the potential effect of coadministered inhibitors on the total exposure of the new drug. Drugs that are not significantly cleared by metabolism (e.g., $f_m<0.3$) will not suffer much when coadministered with an inhibitor of that enzyme, regardless of how potent the inhibitor is or the concentration at which it is given or attains. In contrast, a drug that is cleared entirely by metabolism ($f_m=1$) will be markedly affected by inhibition of its clearance, with a severalfold increase in area under the curve possible. Consequently, a drug with a narrow therapeutic index that is metabolically cleared by several P450s is considerably safer than a drug principally cleared by only one P450. It is possible to determine reaction phenotyping (i.e., $f_m$) after the completion of an antibody inhibition study or any of the other approaches. By knowing the enzymes involved in metabolic clearance and their relative contribution, one can simulate the potential effect of inhibition on exposure and safety.

Table 1 summarized applications of P450-raised antibodies in P450 research. Because P450s are responsible for the clearance of approx-
References


