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HUMAN EXTRAHEPATIC CYTOCHROMES P450: Function in Xenobiotic Metabolism and Tissue-Selective Chemical Toxicity in the Respiratory and Gastrointestinal Tracts*

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■ **Abstract** Cytochrome P450 (CYP) enzymes in extrahepatic tissues often play a dominant role in target tissue metabolic activation of xenobiotic compounds. They may also determine drug efficacy and influence the tissue burden of foreign chemicals or bioavailability of therapeutic agents. This review focuses on xenobiotic-metabolizing CYPs of the human respiratory and gastrointestinal tracts, including the lung, trachea, nasal respiratory and olfactory mucosa, esophagus, stomach, small intestine, and colon. Many CYPs are expressed in one or more of these organs, including CYP1A1, CYP1A2, CYP1B1, CYP2A6, CYP2A13, CYP2B6, CYP2C8, CYP2C9, CYP2C18, CYP2C19, CYP2D6, CYP2E1, CYP2F1, CYP2J2, CYP2S1, CYP3A4, CYP3A5, and CYP4B1. Of particular interest are the preferential expression of certain CYPs in the respiratory tract and the regional differences in CYP expression profile in different parts of the gastrointestinal tract. Current research activities on the characterization of CYP expression, function, and regulation in these tissues, as well as future research needs, are discussed.

OVERVIEW

Cytochrome P450 (CYP) enzymes, particularly those in the *CYP1*, *CYP2*, and *CYP3* gene families (1), catalyze the biotransformation of a wide variety of xenobiotic compounds. The organ that expresses the highest levels of CYP is the liver,

*Abbreviations used in text: CYP, cytochrome P450; HMPA, hexamethylphosphoramide; NDEA, N-nitrosodiethylamine; NNK, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; CPR, NADPH-cytochrome P450 reductase; RT-PCR, reverse transcriptase-polymerase chain reaction; TCDD, 2,3,7,8-tetrachlorodibenzo-p-dioxin; AFB1, aflatoxin B1; BaP; benzo(a)pyrene; G.I., gastrointestinal.

which plays the dominant role in the first-pass clearance of ingested xenobiotic compounds and controls the systemic level of drugs and other substrate chemicals. Extrahepatic tissues, especially those that are the portals of entry for foreign compounds, such as the respiratory and the gastrointestinal tracts, also express xenobiotic-metabolizing CYPs. In these tissues, CYPs not only contribute to the first-pass clearance but may also influence the tissue burden of foreign compounds or bioavailability of therapeutic agents.

CYP-mediated drug metabolism can also lead to altered drug efficacies through inactivation of an active drug or activation of a prodrug. Because most drugs have their targets in extrahepatic tissues, the extent and characteristics of target-tissue drug metabolism may have a significant impact on effectiveness of treatment. By the same token, toxic compounds may be detoxified following CYP-catalyzed biotransformation, and inert xenobiotics, including drugs, may be activated to become toxicants. Most xenobiotic compounds require metabolic activation by CYPs to form ultimate carcinogens or toxicants. The reactive intermediates resulting from CYP-catalyzed metabolic activation are often unstable and therefore are unlikely to be transported from the liver to other tissues to exert toxicity. Thus, chemical toxicity in extrahepatic tissues frequently results from in situ metabolic activation mediated by CYPs in the target organ, and the toxicity of a given compound is tightly linked to its metabolic fate in the target tissue.

Each tissue has a unique profile of CYP enzymes that, by and large, determine the sensitivity of that organ to a given xenobiotic compound. Although most CYPs expressed in extrahepatic tissues are also present in the liver, and often at higher levels, at least some of them may be regulated differently in different tissues, therefore leading to a tissue-selective response to chemical exposure. Furthermore, some CYPs are expressed preferentially in extrahepatic tissues, which may lead to unique extrahepatic metabolites and tissue-specific consequences in cellular toxicity and organ pathology. Risk assessment of potential human toxicants is currently based primarily on data obtained from animal bioassays and on knowledge of the mechanism of toxicity derived from experimental animals. However, because of the well-known species differences in biotransformation, a detailed characterization of the expressed CYP enzymes in human target tissues is crucial for a more accurate prediction of human risk.

Tremendous progress has been made in recent years in the characterization of extrahepatic-tissue CYP expression, function, and regulation. The scope of this review does not allow discussion of all extrahepatic tissues or studies on all species. We have chosen to focus on the human respiratory and gastrointestinal (G.I.) tracts because of their importance as portal-of-entry organs in xenobiotic metabolism and because of our active research in these areas. Human *CYP* genes expressed in different parts of the respiratory and G.I. tracts are summarized in Table 1.

TABLE 1 Human cytochrome P450 genes expressed in different parts of the respiratory and gastrointestinal tracts[a]

Organ	CYPs detected[b]
Nasal mucosa	2A6, 2A13, 2B6, 2C, 2J2, 3A
Trachea	2A6, 2A13, 2B6, 2S1
Lung	1A1, 1A2, 1B1, 2A6, 2A13, 2B6, 2C8, 2C18, 2D6, 2E1, 2F1, 2J2, 2S1, 3A4, 3A5, 4B1
Esophagus	1A1, 1A2, 2A, 2E1, 2J2, 3A5
Stomach	1A1, 1A2, 2C, 2J2, 2S1, 3A4
Small intestine	1A1, 1B1, 2C9, 2C19, 2D6, 2E1, 2J2, 2S1, 3A4, 3A5
Colon	1A1, 1A2, 1B1, 2J2, 3A4, 3A5

[a]See text for references.

[b]Either mRNA or protein.

CYTOCHROME P450 IN THE RESPIRATORY TRACT

Introduction

Tissues of the respiratory tract, which are exposed to both inhaled and blood-borne xenobiotic compounds, are important targets for environmental toxicity. The nasal mucosa is a "metabolic hot spot" in animals. Nasal tumors and other nontumor toxic effects are readily induced in experimental animals following inhalation or systemic exposure to a variety of industrial chemicals, environmental pollutants, therapeutic agents, and cigarette smoke-associated chemicals including the tobacco-specific nitrosamines [for a review, see (2)]. The incidence of human nasal tumors is generally low, although it is a common cancer in parts of China. An increased incidence of nasal tumors is found in smokers and in persons occupationally exposed to wood dust, chromate, and other chemicals (2), and extensive DNA damage is found in the nasal epithelium of children exposed to urban pollution (3). Numerous compounds have also been found to cause toxicity in the lung [for reviews, see (4, 5)]. In humans, lung cancer is the leading cause of cancer-related death in the United States, and cigarette smoking is the most important contributing factor to lung cancer (6, 7). In addition, possible links between respiratory tract xenobiotic metabolism and the etiology of asthma as well as multiple chemical sensitivity are important topics for exploration. Thus, characterization of biotransformation enzymes in human nasal mucosa and lung is important for risk assessment of potential respiratory tract toxicants.

Many cell types within the lung, such as the bronchial epithelial cells, Clara cells, type II pneumocytes, and alveolar macrophages, are capable of metabolizing xenobiotics. In rodents and rabbits, however, Clara cells and type II cells are the

most active [for reviews, see (8, 9)]. Accordingly, these cell types are also more susceptible than other cell types to toxicities resulting from the metabolic activation of xenobiotics. In humans, the Clara cells may be less important as a target tissue for metabolic activation of xenobiotics because they seem to lack smooth endoplasmic reticulum (4). In the nasal mucosa, the microsomal CYPs are expressed in non-neuronal cells, including the sustentacular cells in the olfactory epithelium and cells of the Bowman's glands in the submucosa (10).

Most studies on xenobiotic-metabolizing CYPs in the respiratory tract focus on the role of these enzymes in metabolic activation and toxicity. Little is known of the roles of human respiratory tract CYPs in the disposition or efficacy of therapeutic agents despite an increased interest in using the nose as an alternative or even preferred routed of drug delivery. Nevertheless, the knowledge gained from the basic characterization of the respiratory tract CYPs and the biological models established for studying the role of these CYPs in metabolic activation and portal-of-entry organ xenobiotic toxicity will facilitate future studies on their possible roles in local drug clearance and efficacy.

The subject of respiratory tract CYPs has been covered in several previous reviews [e.g., (2, 11–13)] and by a comprehensive monograph (14). Therefore, only recent advances are reviewed here, with an emphasis on CYPs expressed preferentially in the respiratory tract. Unlike the following section on CYPs in the G.I. tract, this section is not divided according to anatomical parts; the nasal mucosa, lung, and trachea all seem to share a common subset of CYPs that are expressed preferentially in the respiratory tract.

Expression of Cytochrome P450 in the Respiratory Tract

Many microsomal CYPs have been detected in human lung, including CYP1A1, 2B6, 2E1, 2F1, 3A4, 3A5, 4B1 (13), CYP1A2 (15), 1B1 (16, 17), 2A6 (18, 19), 2A13 (20), 2C (18, 21), 2D6 (22), 2J2 (23), and 2S1 (24). Most of these enzymes are expressed in the lung at levels much lower than in liver, but several, including CYP2A13 (20), 2F1 (25), 2S1 (24), 3A5 (26), and 4B1 (27), are preferentially expressed in the lung. CYP2A6, 2A13 (20), 2B6 (28), and 2S1 (24) have also been detected in the trachea.

Several studies have examined the expression of CYPs in human nasal mucosa. CYP2A6 (29), 2A13 (20, 30), 2C, and 3A (31) have been detected in adult nasal mucosa, and CYP2A6, CYP2A13, CYP2B6, and CYP2J2 have been detected in fetal nasal mucosa (32). Additional CYPs are expected to be present in human nasal mucosa because more than 10 different microsomal CYPs, including CYPs of the 1A, 2A, 2B, 2C, 2E, 2G, 2J, 3A, 4A, and 4B subfamilies, have been identified in the nasal mucosa in various animal species (33). Notably, CYP content in the nasal mucosa is among the highest of all extrahepatic tissues in many mammalian species, although apparently not in humans (2, 12).

Interestingly, all functional *CYP* genes in a *CYP2* gene cluster on chromosome 19, including *CYP2A6*, *2A13*, *2B6*, *2F1*, and *2S1* (34), are expressed in the

respiratory tract. Moreover, whereas CYP2A6 is expressed primarily in the liver [e.g., (20)] and CYP2B6 is expressed in liver and many other tissues (28), CYP2A13, 2F1, and 2S1 are preferentially expressed in the respiratory tract. These latter genes, which are particularly interesting because of their likely roles in tissue-selective chemical toxicity, are described in more detail below. Notably, the *CYP2G1* gene, which is expressed only in the olfactory mucosa and is functional in other mammals, has apparently been inactivated in humans (35), and the *CYP2B7P* gene, which is expressed in the lung, is also nonfunctional.

Three full-length genes are known in the human *CYP2A* subfamily (34): *CYP2A6*, *CYP2A7*, and *CYP2A13*. CYP2A6 has been detected in human nasal mucosa (29) and lung (18, 19). CYP2A7, which is nonfunctional (34), was not detected in human nasal mucosa or lung (19, 30). CYP2A13 mRNA is expressed at its highest levels in the nasal mucosa, followed by the trachea and lung (20). The level of CYP2A13 mRNA is much higher than that of CYP2A6 in the respiratory tract.

The expression of CYP2A protein in human lung was reported in an immunoblot study in which a polyclonal anti-CYP2A6 antibody was used (18); this antibody most likely cross-reacts with the highly homologous CYP2A13. More recent studies involving in situ hybridization suggest that CYP2A13 is expressed in both bronchial and alveolar epithelia (J. Guo & X. Ding, unpublished data). Abundant expression of CYP2A proteins in both olfactory and respiratory nasal mucosa was demonstrated in an earlier immunohistochemical study (10). A CYP2A13-specific antibody is not yet available. Heterologously expressed CYP2A13 is active toward many compounds (20), such as 2′-methoxyacetophenone, 2,6-dichlorobenzonitrile, hexamethylphosphoramide (HMPA), *N*,*N*-dimethylaniline, *N*-nitrosodiethylamine (NDEA), and *N*-nitrosomethylphenylamine. CYP2A13 also appears to be the most efficient CYP enzyme in the metabolic activation of a well-known tobacco-specific carcinogen, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK).

There is only one functional gene in the human *CYP2F* subfamily. CYP2F1 was originally cloned from a human lung cDNA library (25). Substrates for CYP2F1 include ethoxycoumarin, propoxycoumarin, and pentoxyresorufin, but not ethoxyresorufin (25). Heterologously expressed CYP2F1 had only modest activity in the metabolic activation of 4-ipomeanol, a pulmonary toxin (36), but it was efficient in the metabolic activation of several other lung toxins, including 3-methylindole (37), naphthalene (37), and styrene (38). The relative rates of metabolic activation of 3-methylindole by CYP2F1 and CYP2A13 have not been examined, but CYP2F1 was approximately three times more active than was CYP2A6 in this reaction (39).

CYP2S was recently identified through a bioinformatics approach; the full-length cDNA was obtained by reverse transcription-polymerase chain reaction (RT-PCR) (24). CYP2S1 mRNA appears to be highly expressed in trachea and lung, and CYP2S1 protein was also detected in human lung by Western blot analysis using an antiserum against the C terminus of the enzyme. However, the activity of the enzyme was not reported. Of interest, CYP2S1 was inducible

by 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in a human lung epithelial cell line (40).

Because of potential vulnerability of fetuses to developmental toxicity resulting from transplacental xenobiotic exposure, several studies have examined the expression of CYPs in various fetal tissues. CYP1A1 mRNA was detected in fetal lung at gestational days 55 to 145 (41). CYP1B1 mRNA and proteins immunochemically related to CYP3A were also detected in fetal lung (42). Expression of several CYPs, including CYP2A6, CYP2A13, CYP2B6, and CYP2J2, was detected in human fetal nasal mucosa in a recent study (32). The expression of CYP2A13, an avid enzyme for metabolic activation of NNK, in human fetal nasal mucosa may have significant implications in developmental toxicology because NNK can be transferred transplacentally from women who smoke cigarettes to fetuses (43).

Several studies have compared the expression level of CYPs in tumor versus nontumor tissues in the lung [e.g., (44)]. These data may provide useful information for cancer chemotherapy, but may not indicate a role of these CYPs in the initiation of tumorigenesis. The CYP expression levels may change at different stages of tumor development.

Role of Individual Cytochromes P450 in Microsomal Metabolism

Studies on the roles of various human CYPs in the metabolic activation of xenobiotics in the respiratory tract are of paramount importance for understanding the mechanisms of chemically induced nasal and pulmonary toxicities, yet only limited progress has been made. Although CYP-dependent xenobiotic-metabolizing activities have been detected in human nasal microsomes [e.g. (45)], the role of individual CYPs has not been examined. The very limited availability of human nasal tissues for metabolic studies remains a major obstacle. In contrast, human lung microsomes are now readily available for in vitro studies, though these microsomes usually have very low metabolic activity. It is difficult to assess the extent to which in vitro data reflect activities in intact lung in vivo because the level and activity of pulmonary CYPs may be highly sensitive to the surgical conditions to which most lung biopsy or autopsy tissues were exposed. Nevertheless, in vitro studies are still valuable, particularly when combined with other approaches, including the quantification of the levels of enzyme expression in the same microsomal preparations used for metabolic studies and kinetic analysis of heterologously expressed CYPs.

CYP1A1 plays a major role in the metabolic activation of many aromatic hydrocarbons in human lung, such as 5-methylchrysene and 6-methylchrysene (46). Several CYPs, including CYP2F1, CYP4B1, and CYP2A6, may be involved in the metabolic activation of 3-methylindole in human pulmonary microsomes (47). The metabolic activation of aflatoxin B1 (AFB1) in human lung may involve CYP3A4 (48). The metabolism of 1,1-dichloroethylene to a reactive epoxide by human lung microsomes may be mediated by CYP2E1 (49).

Human lung microsomes are active in the metabolism of NNK. However, the major CYPs responsible for NNK activation have not been identified. In lung microsomes, CYP2A6 or a related enzyme was implicated in NNK activation; lipoxygenases and lipid hydroperoxides may also be involved (50). The high efficiency of CYP2A13 in NNK metabolic activation and the preferential expression of CYP2A13 in the respiratory tract (20) suggest that CYP2A13 may play a major role in the metabolic activation of this important respiratory tract procarcinogen. However, direct evidence for its involvement has not been demonstrated at the microsomal level.

NNK metabolism has not been examined in human nasal mucosa. However, human nasal microsomes were found to have relatively high activities in the N-deethylation of NDEA (45). Metabolic activation of NDEA has also been demonstrated in human lung cell lines (51). However, the CYPs responsible for the metabolic activation of NDEA in human nasal mucosa and lung have not been identified. Human nasal mucosa is also active in the metabolic activation of HMPA, a known rodent nasal carcinogen (45). Both CYP2A6 and CYP2A13 are active in this reaction, with CYP2A13 being the more active of the two (20).

Biological Models for Studying Function of Respiratory Tract Cytochromes P450 in Xenobiotic Metabolism and Chemical Toxicity

Metabolic studies using biological models are important for determining the significance of specific enzymes or pathways in toxicity. For example, in an interesting series of studies (52), murine lung tumors were found to have diminished CYP expression and activities, and they were also found to be resistant to cytotoxicity induced by xenobiotics, thus supporting a role of local CYP in metabolic activation. However, to date no studies have directly evaluated the in vivo role of human respiratory tract CYPs in xenobiotic metabolism or toxicity. Limited studies have been done with animal models or cell lines. For example, Mace and coworkers (53) transfected human CYP1A2 into a human lung cell line and demonstrated increased cytotoxicity following exposure of the cells to AFB1. The abilities of specific lung cells to metabolize toxicants were also demonstrated for isolated lung cells of different types (54). Other models include microdissected airway cultures, which maintain their differentiated status and CYP expression (55).

Studies correlating the levels of benzo(a)pyrene (BaP)-DNA adduct in human lung with *CYP1A1* genotype have also been reported (56). Although these studies do not necessarily indicate the role of lung CYPs in metabolic activation, a recent study did confirm the role of CYP1A1 induction in BaP-DNA adduct formation in two human lung tumor cell lines (57). The potential correlation between genetic polymorphisms in *CYP* genes, such as *CYP1A1*, *CYP2D6*, or *CYP2E1*, and lung cancer incidence has also been studied [e.g., see (7)]. However, a clear and conclusive correlation has not been found with any of the *CYP* genes studied. An interesting development along this line is the somewhat controversial finding that

reduced hepatic clearance of nicotine, as a consequence of genetic polymorphisms in the *CYP2A6* gene, is associated with a lower incidence of lung cancer in certain populations (58). Notably, the *CYP2A13* gene is also highly polymorphic [(59); X. Zhang & X. Ding, unpublished data]. Thus, individuals defective in both *CYP2A6* and *CYP2A13* genes may be further protected from cigarette smoking-induced respiratory toxicity.

An increasing number of animal studies, utilizing *CYP*-knockout mice or utilizing chemical inhibitors, have dealt with the role of CYPs in respiratory toxicity. Again, studies measuring in vivo systemic clearance generally do not provide data regarding specific extrahepatic organ contribution. Although several *CYP*-knockout and transgenic mouse models have been generated [for a review, see (60)], mice with tissue-specific *CYP* gene deletion or respiratory tract–selective expression of human *CYP* transgene have not been reported. However, the *Cyp1a1*-null mice are suitable for conditional gene targeting (61) and therefore should be useful for studying specific roles of CYP1A1 in extrahepatic tissues. Other models, including mice with tissue-specific NADPH-cytochrome P450 reductase (*CPR*) gene deletion, under development in this (X. Ding, unpublished data) and other (62) laboratories may also be highly useful, as they will allow analysis of the combined roles of all microsomal CYPs in a given organ. A potential problem with in vivo studies in mice, however, is the existence of the well-known, yet poorly characterized, strain-related differences in gene expression and function. Furthermore, potential complications resulting from the genetic manipulations involved in the production of a mutant mouse strain may also lead to unexpected results. For example, the expression of the neighboring *Cyp2a5* gene was suppressed in the liver of *Cyp2g1*-null mice (X. Zhuo & X. Ding, unpublished data).

Two studies have examined the effects of *CYP* gene deletion on nasal xenobiotic toxicity. Whereas systemic deletion of *Cyp2e1* protected nasal mucosa from chloroform toxicity (63), deletion of *Cyp1a2* did not protect the nose from acetaminophen toxicity (64). In the latter case, acetaminophen activation by nasal mucosa-selective CYP2A5 and 2G1 was thought to be responsible for the local toxicity. The same may apply to many other compounds known to cause nasal mucosa-selective toxicity, such as coumarin, 2,6-dichlorobenzonitrile, and HMPA. Clearly, knockout models for these nasal mucosa-predominant CYPs should be invaluable for determining whether the nasal CYPs are responsible for tissue-selective toxicity. In the case of CYP2G1, for which humans do not have a functional ortholog, the *Cyp2g1*-null mice also represent a humanized mouse model that may be useful for risk assessment.

Regulation of Cytochromes P450 in Human Respiratory Tract

Several CYPs, including CYP1A1, 1A2, 1B1, 2E1, and 3A5, may be inducible in human lung. For example, CYP1A1, which is normally expressed in the lung at trace levels, is induced significantly by cigarette smoking [e.g., (65)]. Induction of CYP1A1 and 1A2 by TCDD and other compounds was also suggested by a recent

study using human lung explant culture system (66). Hukkanen and coworkers (67) observed that in the human alveolar type II cell-derived A549 adenocarcinoma cell line, CYP1A1 and 1B1 mRNAs were induced 56-fold and 2.5-fold, respectively, by TCDD; CYP3A5 mRNA was induced 8-fold by dexamethasone and 11-fold by phenobarbital, whereas CYP3A4 was not detected. The mRNAs of several other CYPs, including CYP1A2, 2A6, 2A7, 2A13, 2F1, and 4B1, were also not detected in this study. The pulmonary inducibility of CYP2A6, 2B6, 2C, and 3A4, which are all inducible in hepatocytes, has not been documented. Inducibility of CYPs has not been examined in human nasal mucosa, although it has been suggested that potential induction of nasal CYPs by tobacco smoke may enhance resistance to xenobiotics implicated in parkinsonism and other neurologic diseases (68).

Studies correlating levels of CYP expression (mostly of mRNA) with amount of cigarette smoking have been reported, but these are complicated by issues including incomplete accounting of patient exposure and medical history (such as the extent of use of a respirator during surgery), specimen quality (postmortem time and specimen storage condition), heterogeneity of the tissues assayed in which different cell populations may undergo different responses, and potential genetic polymorphisms in *CYP* gene regulation. In vivo studies using noninvasive marker substrates have limited application for extrahepatic tissues due to difficulties in determining the organ distribution of metabolism, but they may hold promise for CYPs expressed preferentially in the respiratory tract, such as CYP2A13. In this regard, it should be feasible to detect and quantify volatile metabolites from inhaled CYP2A13 substrates via techniques such as real-time gas chromatography–mass spectrometry of exhaled air. For some CYPs, such as CYP2E1, the inducibility in extrahepatic tissues can be predicted according to the mechanisms of the CYP's induction, but the extent of its induction will be affected by the concentration of the inducers in the respiratory tract.

The tissue-restricted inducibility of some CYPs, such as CYP2A (69) and CYP2B (11), has been demonstrated in animal models. From a physiological point of view, each CYP may have unique, yet unknown, functions in a tissue, and thus there may be a need for the tissue to maintain a relatively constant level of its expression. Alternatively, some CYPs in the respiratory tract may not respond to ingested or systemically administrated inducers because the level of inducers and duration of exposure cannot be maintained. In addition, an important issue to consider when studying CYP induction in the respiratory tract is that many inducers may cause tissue-selective toxicity, including inflammatory responses, leading to the loss of CYP expression. Thus, a lack of observed induction may not necessarily indicate the existence of tissue-specific transcriptional or posttranscriptional regulatory mechanisms.

The tissue-selective expression of several *CYP* genes in the respiratory tract has been explored in order to identify mechanisms of regulation, primarily through the use of animal models. For *CYP2A*, a nuclear factor I-like element (called NPTA element) is present in the proximal promoter region; it is conserved in rodent and

human *CYP2As* (70). The intriguing possibility that the *CYP2A*, *2B*, *2F*, and *2S* genes, which are part of the chromosome 19 *CYP2* gene cluster, may share common regulatory mechanisms for selective expression in the respiratory tract remains to be explored. The lack of cell lines that normally express these CYPs renders it very difficult to perform traditional promoter analysis. It is also challenging to perform in vitro DNA-binding studies with nuclear extracts from human tissues. A transfection experiment with human lung cells (71) showed that C/EBP factors are needed for differentiation-dependent expression of rat 2B1 in A549 cells; this experiment used a construct previously shown to lead to lung-specific expression in transgenic mice.

The promoter region of human *CYP2F1* is being explored through in vitro methods, with nuclear extracts from human lung (G. Yost, personal communication). An apparently lung-selective binding site was identified within a 31-bp sequence (−152 to −182) in the *CYP2F1* promoter that does not match any known regulatory motif. The same group also evaluated the regulation of the human *CYP4B1* gene and identified two regulatory enhancer domains in the proximal region of the *CYP4B1* promoter, which appear to be unique, previously uncharacterized regulatory elements.

Several transgenic mouse studies have attempted to map important promoter regions for selective CYP expression in lung or nasal mucosa. In one, a 1.3-kb fragment of the rat *CYP2B1* promoter was able to confer tissue-selective expression of a reporter gene in lung and liver (72). Similarly, a rat *CYP2A3* transgene with 3.4-kb 5′-flanking sequence was selectively expressed in the nasal mucosa, as well as in other tissues known to express the endogenous mouse *Cyp2a5* gene (73). However, in both cases, the level of transgene expression was quite low. In another study, a 3.6-kb mouse *Cyp2g1* promoter was used to drive the expression of a reporter gene (74). The transgene was expressed only in the olfactory mucosa, consistent with the idea of tissue-specific expression of the *Cyp2g1* gene. Nevertheless, within the olfactory mucosa, the reporter gene was expressed primarily in the duct cells of Bowman's gland, whereas the endogenous *Cyp2g1* gene is expressed primarily in the supporting cells and Bowman's gland. The results from these studies suggest the need to use large DNA inserts to avoid effects of integration site on transgene expression. Other approaches, such as the use of viral vectors, are being explored (X. Ding, unpublished data); these are less time-consuming, although they are often restricted in the amount of DNA sequence that can be included.

CYTOCHROMES P450 IN THE GASTROINTESTINAL TRACT

Introduction

Elucidation of the roles for CYPs in G.I. tract xenobiotic metabolism has been a slow process, particularly in humans. The capability of the small-intestinal mucosa to metabolize xenobiotics, such as tetrahydrocannabinol (75), flurazepam (76), ethoxycoumarin (77), and aryl hydrocarbons (78), was reported as early as

the mid-to-late 1970s. By the late 1980s and early 1990s, the expression of CYP2C, 2D6, and 3A, as well as CPR, in the human small intestine had been reported (79–83). Ethoxycoumarin *O*-deethylase and aminopyrine *N*-demethylase activities were detected in both the colon and the ileum of older patients (84). The early status of CYP-mediated G.I. tract metabolism was summarized in a review (85), and that of small-intestine metabolism was described in a review from our laboratory (86).

Several factors have contributed to the slow rate of progress of research into G.I. tract xenobiotic metabolism. Principal among these are the low levels of expression of the CYPs, particularly relative to hepatic expression levels, and the difficulties in determining functions for the expressed CYPs. Possible roles in protection of the body against orally ingested xenobiotics through limitation of systemic uptake have been proposed, as have roles in carcinogenesis through target-organ bioactivation.

In recent years, interest in G.I. tract metabolism, particularly small-intestinal metabolism, has exploded. The resultant studies over the past 10 years are reviewed here.

Anatomy and Physiology of the Gastrointestinal Tract

For the purposes of this review the G.I. tract is defined as comprising the esophagus, stomach, small intestine (subdivided into the duodenum, jejunum, and ileum), and the colon. The tract serves as the portal of entry for orally ingested xenobiotics, including therapeutic drugs and nutrients.

The gross structures of the luminal surfaces of the G.I. tract components are essentially similar, covered with a layer of columnar epithelial cells, goblet cells, and endocrine cells (87). However, the cell types vary among organs. In the stomach, the cells are specialized for the secretion of acid, pepsinogen, gastrin, and intrinsic factor. In the small intestine, the epithelial cells are sited along the villi and microvilli and contain digestive enzymes, transport mechanisms, and metabolic enzymes, including CYPs (see below). The colonic epithelial cells function primarily to absorb fluid and electrolytes. The average lengths and absorbing surface areas of the G.I. tract organs, determined post mortem, are: esophagus, 25 cm and 0.02 m^2; stomach, 20 cm and 0.11 m^2; duodenum, 25 cm and 0.09 m^2; jejunum, 300 cm and 60 m^2; ileum, 300 cm and 60 m^2; and colon, 150 cm and 0.25 m^2, respectively (88, 89). The bacterial floral counts in the various organs show considerable variation: stomach, 0–5 $\log_{10}$ number of viable organisms per g wet weight; proximal small intestine, 0–5; distal small intestine, 6–7; and large intestine, 7–10 (90). Because the presence of gut flora can confound metabolic studies of the G.I. tract, these variations in content are important for investigations of G.I. tract CYP function.

Cytochrome P450 Expression in the Esophagus

The human esophagus is a target organ for cancer, and this has potentiated searches for metabolic bioactivators of tobacco-smoke carcinogens in the organ. Human

esophageal microsome preparations, obtained from individuals in the United States and China, all activated *N*′-nitrosonornicotine (91), as did esophageal cultures (92). Rates of bioactivation were higher in tissue from those Chinese patients who resided in an esophageal cancer high-risk area. This activity was decreased by 20%–26% in the esophageal microsomes by troleandomycin, a CYP3A inhibitor (91). A role for CYP2A6 was ruled out because of the lack of coumarin inhibition found. Additionally, for esophageal squamous-cell carcinomas, levels of what was presumed to be CYP3A4 were 30%–50% decreased, relative to the levels in the surrounding noncancerous tissue. Low levels of CYP2E1 were also detected in these microsomes through immunoblotting. Another nitrosamine, *N*-nitrosomethyl-*N*-amylamine, was also metabolized by esophageal CYPs (93).

The expression of CYP3A in the esophagus was confirmed at the mRNA level (94). Subsequently, in a comprehensive study of esophageal CYP expression, it was resolved by RT-PCR that only CYP3A5, and not CYP3A4, was expressed in all of 25 non-neoplastic surgical samples (95). This preference for expression of one or the other of the two CYP3As is an interesting feature of the G.I. tract and is discussed in more detail below. In the same study, a combination of RT-PCR and immunoblots using specific antibodies revealed the expression of CYP1A, 2E1, and 4A in the esophagus (95). CYP4B1 mRNA was also detected, as was a protein that cross-reacted with anti-CYP2A. CYP1A2 mRNA was detected in 11 of 19 samples, but expression of the protein was not confirmed. CYP1A1 was detected by immunoblot analysis and enzyme assays in all of 41 samples from squamous-cell cancer patients (96). Tumor tissue expressed higher levels of CYP1A1 protein than did normal tissue, but levels were apparently not influenced by the smoking behavior of the patients.

The detection of expressed CYP2E1 in the esophagus prompted a study to determine whether the C1/C1 variant *CYP2E1* genotype would affect the susceptibility of an individual to esophageal cancer (97). This genotype, when studied jointly with the glutathione transferase GSTM1 non-null genotype, showed an odds ratio of 8.5, suggesting that CYP2E1 plays a role in development of esophageal cancer. However, a subsequent study in China did not detect any relationship between the *Rsa* I homozygous *CYP2E1* genotype and esophageal cancer (98). The recently identified CYP2J2 is expressed most prominently in the esophagus among all G.I. tissues (99). The enzyme, which is an arachidonic acid epoxygenase, is speculated to be involved in neuropeptide release.

Cytochrome P450 Expression in the Stomach

There is very limited evidence for CYP expression in the human stomach. Furthermore, it is difficult to propose any function for gastric CYPs because the gastric epithelium secretes rather than absorbs. However, the potential of those CYPs expressed in the stomach to play roles in stomach cancer has been investigated in cases of intestinal metaplasia of the stomach. Intestinal metaplasia of the stomach, which involves the replacement of the gastric mucosa with a small intestine-like epithelium (100), is considered to be a precancerous lesion (101). A combination

of immunohistochemistry, immunoblotting, and RT-PCR have identified CYP3A4 in the foveolar or pitted epithelium of the stomach and in the pyloric gland when intestinal metaplasia is present, but not in its absence (31, 102). These authors also detected expression of CYP2C, by immunoblotting, in gastric fundic glands.

Similar results were obtained with immunoblot and RT-PCR probes for CYP1A1 and 1A2 (103). CYP1A1 and 1A2, as well as CPR, were reported to be detected in human gastric mucosa with intestinal metaplasia and in pyloric gland cells. Microsomes prepared from these cells activated BaP and 2-amino-2-methylimidazole [4,5-f]quinoline.

In view of the precancerous nature of intestinal metaplasia, it has been postulated that the coincident expression of various CYPs in the gastric mucosa of such patients plays a role in the bioactivation of gastric carcinogens. In a comparison of normal stomach tissue with stomach cancer, it was determined that in the normal tissue, no CYPs were detected, whereas in the case of stomach cancer, CYP1A and 3A were detected in 51% and 28% of cases, respectively (104). The presence of intestinal metaplasia was not noted in this study.

Other studies have reported expression of CYPs in human stomach mucosa. The recently identified CYP2S1 mRNA was detected by dot blot analysis (24), and CYP2J2 was detected by immunoblot (99).

Cytochrome P450 Expression in the Small Intestine

The significant xenobiotic absorptive function of the human small intestine provides the framework for an enhanced metabolic role for this organ's expressed CYPs, relative to their roles in other G.I. tract organs. Small-intestinal CYP-mediated metabolism can serve as a barrier to the systemic uptake of xenobiotics, including drugs, by facilitating excretion to the lumen of the intestine or by bioactivation of the xenobiotics, with consequent binding to enterocyte macromolecules. Covalently bound xenobiotics will be removed with the sloughed-off enterocytes, which have very short half-lives. These metabolic activities of the small intestine can produce a detoxification by diminishing systemic uptake of toxicants. This barrier activity of small-intestinal CYPs is greatly facilitated, in the case of CYP3A4 substrates, by the multidrug efflux pump P-glycoprotein, which is expressed in the small intestine and functions in accord with CYP3A4 (105–107). It is probable that small-intestinal CYP3A4 contributes substantially to the first-pass metabolism of high-turnover CYP3A4 substrate xenobiotics (108).

The most extensive characterization of human small-intestinal CYP expression was conducted recently using enterocytes eluted from 10 small intestines by an EDTA-containing buffer. This method of enterocyte preparation produces only villous enterocytes, without crypt cell contamination (109). RT-PCR of enterocytes revealed the expression of CYP1A1, 1B1, 2C, 2D6, 2E1, 3A4, and 3A5 mRNAs. Not detected were CYP1A2, 2A6, 2A7, 2B6, 2F1, 3A7, and 4B1 mRNAs. However, when probed by immunoblots, only CYP3A4, 1A1 (in two of eight intestines tested), and 2C proteins were detectable, and CYP1B1, 2E1, 2D6, and 3A5 proteins were not detectable. In a broader study of 33 small intestines, no

CYP3A5 protein was detected (L.S. Kaminsky, unpublished data). The variability of expression determined for CYP1A1 is consistent with the conflicting reports of its expression (110–112). This expression of small-intestinal CYP1A1 is probably inducible rather than constitutive, to judge from its reported induction by omeprazole, as determined through duodenal biopsies. Total microsomal protein content decreased markedly as a function of distance along the intestine, from the duodenum to the ileum. Total CYP content increased slightly in proceeding from the duodenum to the jejunum and then decreased sharply toward the ileum (109). Our observations that CYP3A4 is the predominant small-intestinal CYP3A expressed and that CYP3A5 protein expression is not detectable are at odds with observations of some other investigators. In a study of 20 enterocyte preparations, a band on immunoblots in four of the samples was indicated to represent CYP3A5, but no positive identification was provided (113). In an earlier study, 14 of 30 patients were reported to express just-detectable levels of CYP3A5 in intestinal biopsies, which were probed with an antibody that was claimed to be specific for CYP3A5; however, no data were presented (114). A clear conclusion is that, at the very least, CYP3A4 expression greatly predominates over that of CYP3A5 in human small-intestinal enterocytes.

The determination of CYP2C protein expression in the small intestine (109) confirmed the results of an earlier study (82). In a subsequent study, metabolic activities were assayed to determine which forms of CYP2C were expressed in the human small intestine and to assess the interindividual variability in expression levels (115). Expression of CYP2C9 and that of CYP2C19 were demonstrated by activities of diclofenac 4′-hydroxylase and mephenytoin 4′-hydroxylase, respectively. Interindividual variability for the 10 intestines investigated was 18-fold for CYP2C9 and 17-fold for CYP2C19. On the basis of 6β-testosterone hydroxylase activity, CYP3A4 activities varied sevenfold for these 10 small-intestinal preparations, although in larger populations much greater variability has been observed. The basis for such variability probably resides in the pathways of regulation of *CYP3A4*, rather than in genetic polymorphisms leading to structural CYP protein variants (116, 117).

Several other CYPs are reported to be expressed in the human small intestine. These include CYP2S1 (24); CYP4F12, which catalyzes the antihistaminic ebastine's metabolism (118); and CYP2J2, which catalyzes arachidonic acid metabolism (99).

Observations that one or more constituents of grapefruit juice can decrease the metabolic function of human small-intestinal CYP3A4 have provided an approach to investigate this function in vivo. The original observation was that grapefruit juice, when administered together with either of the calcium antagonists nifedipine or felodipine, increases the plasma concentration of the drug (119). Subsequently, it was demonstrated that the flavonoids naringenin, quercetin, and kaempferol in grapefruit juice inhibit CYP3A4, which could explain the previous observation (120). Quercetin was later excluded as a possible inhibitor (121), and narignin and naringenin were shown not to be the primary inhibitors in grapefruit juice (122). The metabolism of coumarin, cyclosporine, ethinylestradiol, midazolam,

terfenadine, and verapamil [for reviews, see (123, 124)], as well as that of saquinavir (125, 126) and erythromycin (127), were also shown to be decreased by grapefruit juice.

The grapefruit-mediated decrease in substrate metabolism was determined to be through a mechanism-based inactivation of enterocyte CYP3A4, possibly by a furancoumarin constituent of grapefruit juice (128). Through the use of small-intestinal biopsies and an erythromycin breath test, it was determined that orally ingested grapefruit juice did not affect hepatic CYP3A4 activity but did decrease small-intestinal CYP3A4 levels by 62%, without any corresponding change in the enterocyte CYP3A4 mRNA levels (110). These results are consistent with the proposed mechanism. A recent study showed that at least six furano-coumarins in grapefruit juice contribute to the inhibition of CYP3A4 and that a combination of competitive and mechanism-based inhibition occurs (129).

Cytochrome P450 Expression in the Colon

Interest in expression of CYPs in the colon has been stimulated by the prominence of the colon as a target organ for cancer. Despite this, few recent studies have been reported, and most studies on CYP regulation have been conducted in cell preparations.

No exhaustive studies of CYP expression profiles in the colon have been published. The most prominent CYP expressed is CYP3A, and there is some disagreement concerning which members of this subfamily are actually expressed. As indicated previously, the test systems, e.g., immunoblots or RT-PCR, must be capable of differentiating between the forms to resolve whether CYP3A4 or 3A5 is being expressed. Furthermore, the detection of mRNA for one or the other of the two CYP3As does not necessarily imply that the corresponding protein will be expressed at high-enough levels to be detectable by immunoblot analysis.

Testing by RT-PCR of biopsy tissue from five colons identified the expression of CY3A3 (considered to be an artifact and to be indicative of CYP3A4), CYP1A1, and CYP1A2 mRNAs (130). This contrasts with an earlier study, in which the CYP1A subfamily was not detected (131). However, in this early study, CYP3A was detected at the mRNA level in some individuals and not in others. When CYP3A mRNA was detected in an individual colon, it was observed throughout the length of the organ. In a study of 11 human colons, Northern blot analysis revealed marked interindividual variations in CYP3A mRNA levels, and two species of mRNA were detected in some individuals. CYP3A4 was detected in 5 of the 11 colon samples, and CYP3A5 in 6 of the 11 colon samples (111). In a carefully controlled study in which isoelectric focusing was used to resolve the various CYP3A members, it was determined that CYP3A5 was the "main" form expressed in the human colon at both the mRNA and protein levels (132). In this study, patients were treated with rifampicin. The arachidonic acid epoxygenase, CYP2J2, is also expressed in the colon and presumably plays roles in vascular tone and motility (99).

It has also been reported that CYP1B1 is expressed at high frequency in colon tumors but not in normal colon tissue (133). This result needs to be confirmed

because the same authors have made similar claims, which were not verified, for breast tissue.

Assessment of induction of human colon CYPs in vivo using biopsy tissue has not been reported. An alternative approach, which uses xenografts of human colon tumor in mice, has revealed that CYP2A, 2B, 2C, 3A, and 4A family and subfamily members are inducible at the transcriptional levels by the prototypic CYP inducing agents 3-methylcholanthrene, β-naphthoflavone, clofibrate, dexamethasone, and phenobarbital (134). Colon mucosal tissue obtained by endoscopic biopsy was used to assess the effects of repeated grapefruit juice ingestion on CYP3A expression levels in the colon (110). Colon levels of CYP3A5 were not affected by the grapefruit juice. This contrasts with the marked decrease in small-intestinal CYP3A4 protein previously discussed.

Human Colon Cell Lines

Human colon adenocarcinoma LS-174 and Caco-2 cells, when treated with low doses of natural indoles such as ascorbigen, show up to 21-fold increases in CYP1A1 levels (135). Natural isothiocyanates did not produce any comparable induction, although they induced CY1A1 mRNA. When benzanthracene, pyrazole, or phenobarbital was added to these cells, each induced CYPs, as determined by analysis for *O*-6-methylguanine DNA adducts with 1,2-dimethylhydrazine (136).

CYP3A4 can be induced in the colon cancer cell line Caco-2 by 1,25-dihydroxyvitamin D-3 (137), and this induction is suppressed by nitric oxide but not by a guanylate cyclase inhibitor or by 8-bromo cGMP (138). These results were interpreted to indicate that the nitric oxide suppression is possibly not mediated by a guanylate cyclase pathway. These cells also express CYP1A1, 2E1, and 3A proteins (139). In a carefully conducted study, the major CYP3A in both Caco-2 and HT29, a human colonic cell line, was reported to be CYP3A5 (132).

The colon carcinoma cell line LS180 is unusual because it can be readily induced for CYP1A2 mRNA and protein by TCDD, 3-methylcholanthrene, and benz[a]anthracene (140). Both CYP1A1 and 1B1 were similarly induced in these cells. In this same cell line, CYP3A4 was reportedly induced by rifampin, phenobarbital, clotrimazole, reserpine, and isosafrole. CYP3A5 expression in these cells was not affected by most of these agents, but it was upregulated by reserpine and clotrimazole (141).

In summary, the major CYP expressed in the human colon is CYP3A5, but CYP3A4 also appears to be expressed in some individuals. Very little information is available on the in vivo regulation of colonic CYPs, although inducing agents have been identified in cell systems.

FUTURE PERSPECTIVE

Despite the recent surge in research activities, we are just beginning to understand the potential for the CYPs in the respiratory and G.I. tracts to play significant roles in xenobiotic metabolism and chemical toxicity. The application of molecular and

genomics approaches, as well as the availability of genetically modified animal models, will tremendously increase our capability for generating data, but it will still be an enormous challenge to use these data to understand the complexity of in vivo situations in humans. In this regard, exciting developments are underway in the development and characterization of various humanized mouse models, but it remains to be seen to what extent we can use these mouse models to study human extrahepatic CYPs, particularly because we are still far from understanding how different humans are from laboratory animals in terms of extrahepatic xenobiotic metabolism. Ultimately, we need to know the extent to which human extrahepatic tissues contribute to drug clearance, and we need to determine the relative importance in chemical toxicity of hepatic clearance versus extrahepatic target tissue metabolic activation.

It will not be long before most of the genetic polymorphisms in human *CYP*s are identified. This resource will be invaluable for determining the role of human CYPs in individual differences in xenobiotic metabolism and chemical sensitivity. However, some issues may prove to be more difficult to resolve, including how to interpret the roles of genetic polymorphisms that affect both hepatic and extrahepatic metabolism, how to phenotype individuals for genetic polymorphisms of extrahepatic *CYP*s, and how to determine the functional consequences of regulatory region SNPs for *CYP* genes expressed predominantly in extrahepatic tissues. To that end, more studies with human tissues and human subjects are clearly needed to determine which enzyme(s) is important for human extrahepatic microsomal metabolism in vitro and in vivo and to understand the extent of xenobiotic inducibility of human extrahepatic CYPs.

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