invited review

The lung HETEs (and EETs) up

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Jacobs, Elizabeth R., and Darryl C. Zeldin. The lung HETEs (and EETs) up. *Am J Physiol Heart Circ Physiol* 280: H1–H10, 2001.—Arachidonic acid metabolites of the cyclooxygenase and lipoxygenase pathways have a variety of important lung functions. Recent observations indicate that cytochrome P-450 (P-450) monooxygenases are also expressed in the lung, localized to specific pulmonary cell types (e.g., epithelium, endothelium, and smooth muscle), and may modulate critical lung functions. This review summarizes recent data on the presence and biological activity of P-450-derived eicosanoids in the pulmonary vasculature and airways, including effects on pulmonary vascular and bronchial smooth muscle tone and airway epithelial ion transport. We hypothesize a number of potential functions of P-450-derived arachidonate metabolites in the lungs such as contribution to hypoxic pulmonary vasoconstriction, regulation of bronchomotor tone, control of the composition of airway lining fluid, and limitation of pulmonary inflammation. Finally, we describe a number of emerging technologies, including congenic and transgenic strains of experimental animals, P-450 isoform-specific inhibitors and inhibitory antibodies, eicosanoid analogs, and vectors for delivery of P-450 cDNAs and antisense oligonucleotides. These tools will facilitate further studies on the contribution of endogenously formed P-450 eicosanoid metabolites to lung function, under both normal and pathological conditions.

arachidonic acid; monooxygenases; eicosanoids; pulmonary arteries; bronchi; bronchospasm; cytochrome P-450; inflammation; hypoxic vasoconstriction; mitogenesis

THE IMPRESSIVE CAPACITY of lung cells to release arachidonic acid from membrane phospholipid stores via the actions of phospholipases (e.g., cytosolic PLA₂), both constitutively and in response to a variety of biological or mechanical stimuli, has been recognized for more than three decades (43). Once released, the free arachidonic acid is available for oxidation along one of three major metabolic pathways: 1) the prostaglandin H synthase (PGHS, cyclooxygenase) pathway, which produces the prostaglandins, thromboxane and prostacyclin; 2) the lipoxygenase pathway, which produces the leukotrienes, midchain hydroxyeicosatetraenoic acids (HETEs) and lipoxins; and 3) the cytochrome P-450 (referred to as P-450 throughout this review) monooxygenase pathway, which produces midchain and ω-terminal HETEs and cis-epoxyeicosatrienoic acids (EETs) (41, 43, 56, 62, 97).

Products of the PGHS pathway have established pharmacological and physiological functions in the lung. For example, prostaglandin E₂, a product of airway epithelium, is a potent bronchodilator and has anti-inflammatory effects in the lung (61, 75). Prostacyclin, a major metabolite of pulmonary artery endothelium, has vasodilatory, bronchodilatory, and platelet antiaggregatory effects (37, 43). In contrast, prostaglandin F₂α and thromboxane are potent bronchoconstrictors, enhance platelet aggregation, and are also vasoconstrictors (43, 61). Recent evidence from PGHS-deficient mice has supported a role for both

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PGHS-1 and PGHS-2 products in countering lung inflammatory responses to inhaled allergen and lipopolysaccharide (30, 111). PGHS-1 products also limit allergen- and lipopolysaccharide-induced bronchial hyperresponsiveness (30, 111). Similarly, a large body of data support the critical role of lipoxygenase products of arachidonic acid in contributing to the airway inflammation, bronchoconstriction, increased mucous secretion, and vascular permeability that accompanies experimental and clinical asthma (1, 22). Inhibitors of the 5-lipoxygenase pathway and leukotriene receptor antagonists are now mainstays in the pharmacological therapy of asthma (21, 22).

A great deal less is known about the role of products of the third pathway of arachidonic acid metabolism, the cytochrome P-450 monooxygenase pathway, in lung physiology and pathophysiology. P-450-derived eicosanoids possess a myriad of biological activities in other organ systems. For example, EETs are primary candidates for endothelium-derived hyperpolarizing factor (EDHF) and are important mediators of endothelium-dependent relaxation in coronary microvessels (7, 27, 72, 76). Recent data suggest that EDHF-induced dilation of coronary arteries may be inhibited by nitric oxide (NO) (69). 20-HETE is the dominant arachidonic acid metabolite of renal cortical tissue, promotes naturiesis, and is one of the most potent constrictors of renal and cerebral arteries identified to date (3, 41, 55). A growing literature supports abundant expression of several different P-450 isoforms in the lung (78, 81, 109, 110, 115). Furthermore, P-450-derived eicosanoid metabolites have recently been shown to be present in lung tissue and bronchoalveolar lavage fluid and to possess potent actions in the airway and pulmonary vasculature at physiologically relevant concentrations (52, 74, 109, 110, 114). Moreover, tools are rapidly emerging that will enable investigators to elucidate the true endogenous functions of P-450 isoforms and their arachidonic acid products in the lung. Thus the purpose of this work is to briefly review what is currently known about the role of P-450-derived eicosanoids in the lung, to suggest potential physiological and/or pathophysiological implications of pulmonary P-450 isoform expression, to propose several working hypotheses as to the function of P-450-derived eicosanoids in the lung, and to identify potential experimental approaches to prove or disprove these hypotheses.

WHAT ARE CYTOCHROMES P-450 AND HOW ARE THEY REGULATED?

Members of the P-450 superfamily encode membrane-bound, heme-containing proteins that catalyze the oxygen- and NADPH-dependent metabolism (oxidation, peroxidation, and/or reduction) of xenobiotics, including industrial and pharmaceutical chemicals and carcinogens (67). For this reason, studies of P-450s have long been the province of toxicologists and pharmacologists. Over the last two decades, investigators have recognized that P-450 proteins also oxidize endogenous lipids, including arachidonic acid, retinoic acid, and linoleic acid. P-450 metabolism of arachidonic acid forms a series of regiospecific and stereospecific fatty acid epoxides (5, 6, 8, 9, 11, 12, and 14-15-EETs) and alcohols (midchain and α-terminal HETEs) (9, 10). In general, the product profile is P-450 isoform specific. Thus some P-450s are primarily arachidonic epoxygenases (e.g., CYP2B, CYP2C, and CYP2J subfamily members), whereas others are primarily arachidonic acid hydroxylases (e.g., CYP4A and CYP4F subfamily members).

Most P-450s are primarily expressed in the liver, with significantly lower levels of expression in extrahepatic tissues (33). Some P-450s, such as members of the CYP2J and CYP4A subfamilies, are predominantly detected in tissues outside the liver, including the heart, vasculature, gastrointestinal tract, kidney, and lung (47, 84, 89, 103, 104, 114). Some P-450 isoforms are expressed constitutively in these tissues, whereas others are induced by xenochemicals. For example, CYP1A1 and CYP2E2 isoforms are rapidly induced by aromatic and simple hydrocarbons, CYP2B isoforms are induced by barbiturates and CYP4A isoforms are upregulated in the liver by hypolipidemic drugs (101, 107). Remarkably little is known regarding the regulation of extrahepatic, and particularly pulmonary, P-450 isoforms by xenochemicals. Domin and co-workers (19, 20) have shown upregulation of pulmonary CYP1A1 in response to aromatic hydrocarbons. In contrast, phenobarbital has effects on pulmonary CYP2B expression despite causing a dramatic induction of this P-450 subfamily in the liver (29). Interestingly, Ohnhaus and Bluhm (71) reported increased P-450 monooxygenase activity in lungs of patients with active tuberculosis treated with rifampicin. Many of these chemical inducers are reported to modulate hepatic P-450 activity by altering mRNA transcription, mRNA stability, protein translation, and/or protein turnover (67, 107); however, the molecular mechanism(s) underlying the regulation of pulmonary P-450s by these xenochemicals remains largely uninvestigated.

CYTOCHROME P-450 METABOLITES ARE PRODUCED BY LUNG MICROSOMES AND ARE PRESENT ENDOGENOUSLY IN LUNG TISSUE

Dramatic pregnancy-induced upregulation of CYP4A4 in rabbit lungs has been recognized for more than 20 years (78). Glucocorticoids and progesterone have both been shown to upregulate 20-HETE formation in female rabbit lungs (48, 57, 59). The conversion of arachidonic acid into 19- and 20-HETE is also observed in peripheral lung microsomes prepared from male rabbits (110, 112, 114). Immunoblots of microsomes prepared from microdissected rabbit lung segments with a polyclonal antibody raised against purified rat liver CYP4A revealed abundant expression of this P-450 subfamily within the airway (47, 114). Immunohistochemical studies of rabbit lungs localize CYP4A protein expression to nonciliated cells of the proximal airways and to capillary endothelial cells...
(58). Human peripheral lung microsomes are known to convert arachidonic acid to 20-HETE and to contain abundant CYP4A immunoreactive protein (5). There is also NADPH-dependent synthesis of 20-HETE from arachidonic acid in rabbit and human airway microsomes (47). Thus there is constitutive and regulated expression of proteins that catalyze the formation of 20-HETE in human and rabbit lung. Little is known regarding the predominant cellular source(s) of 20-HETE in the lung, which specific CYP4A isoforms are most abundant, and the metabolic fate of pulmonary 20-HETE. Whereas functional data from isolated pressurized renal arteries using 20-HETE analogs suggest receptor-dependent vasoactivity in the kidney (2), there is no information regarding the nature, location, or existence of these putative 20-HETE receptors in the lung.

These are also good evidence for the pulmonary epoxidation of arachidonic acid in rabbits, rats, guinea pigs, dogs, and humans (5, 52, 90, 108, 110). Rabbit lung microsomes actively metabolize arachidonic acid to EETs (52, 110). EETs and their hydration metabolites the dihydroxyeicosatetraenoic acids (DHETs) are detectable in rabbit lung homogenates and in bronchoalveolar lavage fluid by gas chromatography-mass spectrometry (110). Northern analysis of human and rat RNA has identified CYP2J transcripts in the lung (103, 104, 108). Immunoblotting studies demonstrate expression of CYP2J subfamily P-450 proteins in rat and human lung (108). Immunohistochemical experiments demonstrate that CYP2J expression is localized to both ciliated and nonciliated airway epithelial cells, bronchial and vascular smooth muscle cells, and endothelium and alveolar macrophages (84, 108). Message for CYP1A2, CYP2B6/7, CYP2E1, CYP2F1, CYP3A5, and CYP4B1 have also been detected in human lung RNA (81). Immunospecific bands for CYP1A, CYP2B, CYP2C, and CYP2E subfamily P-450s are present in rabbit peripheral lung and pulmonary artery microsomes (110, 113). Immuno inhibition studies have demonstrated that CYP2B4 likely contributes to pulmonary P-450 arachidonic acid epoxygenase activity in rabbits (52, 110). Although little information regarding the cellular localization of these P-450 isoforms is available (19, 86), their presence in the lung suggests the possibility that they might at least contribute to EET formation under physiological and/or pathophysiological circumstances. Finally, although there is evidence for surface binding receptor sites for 14R,15S-EET in guinea pig mononuclear cells and U-937 (monocyte line) cells (99, 100), there are no data that address the presence of such binding sites in pulmonary tissue.

**BIOLOGICAL ACTIVITY OF P-450-DERIVED EICOSANOIDS IN THE LUNG**

Data from several laboratories implicate P-450 metabolites as modulators of vascular smooth muscle tone in the cerebral, coronary, and renal vascular beds (3, 7, 27, 31, 55, 72). Although the mechanisms through which P-450 products modulate vascular tone in these tissues are incompletely understood, EETs appear to activate large-conductance, calcium-activated potassium channels in vascular smooth muscle, thereby promoting hyperpolarization of the resting membrane potential and causing vasorelaxation (35). EETs have also been shown to affect L-type Ca\(^{2+}\) channel activity in cardiac muscle cells (13, 105); however, their role in modulating these channels in vascular smooth muscle is unknown. In contrast, 20-HETE potently inhibits calcium-activated potassium channels and activates L-type Ca\(^{2+}\) channels in vascular smooth muscle cells (31). Inhibition of 20-HETE formation by NO is believed to contribute to the vasodilator effects of NO in the renal circulation (4, 93). In addition to vasoactive effects, P-450-derived eicosanoids have been shown to possess a host of other biological effects in nonpulmonary tissues. These include anti-inflammatory effects (70), effects on cellular proliferation (38), ion transport (38, 62), peptide hormone secretion (8, 25), and platelet function (28). Several investigations identifying biological activities and potential functions of P-450 metabolites in the lung are detailed below.

**Effects on Airway Epithelial Ion Transport**

Arachidonic acid has been shown to inhibit mucosal Cl\(^{-}\) secretion in the human airway (42). More recently, epoxygenase metabolites of arachidonic acid were shown to cause concentration-dependent decreases in transepithelial voltage and short-circuit current of rat tracheas and primary cultures of tracheal epithelial cells (74). The changes in transepithelial voltage and short-circuit current were highly enantioselective for 11R,12S-EET, and they appeared to be mediated by a chloride-conductive pathway in that they were blocked by pretreatment with bumetanide (74). Indeed, Salvail and co-workers (83) have shown that EETs inhibit tracheal Ca\(^{2+}\)-sensitive Cl\(^{-}\) currents. Moreover, the P-450 epoxygenase inhibitor ketoconazole has been shown to activate Cl\(^{-}\) conductance in cultured cystic fibrosis cells (50). Together, these data suggest that EETs cause a net reduction in Cl\(^{-}\) secretion by airway epithelium. It remains unknown whether EETs or HETEs affect other lung epithelial ion transport processes.

**Effects on Bronchomotor Tone**

Both 5,6- and 11,12-EETs cause hyperpolarization of the resting membrane potential of rabbit tracheal smooth muscle cells, and they activate large-conductance, Ca\(^{2+}\)-activated K\(^{+}\) currents in lipid bilayer reconstitution experiments (23). These data suggest that the EETs may function as hyperpolarization factors in the airway. In fact, 5,6-EET methyl ester and 8,9-EET have been shown to relax histamine-precontracted guinea pig and human bronchi (110, 115). The bronchomotor effects of 20-HETE appear to be more complicated than those of EETs. 20-HETE causes relaxation of rabbit bronchi precontracted by histamine or KCl, and these effects are inhibited by either denuda-
tion of epithelia or treatment with the PGHS inhibitor indomethacin (47). Similarly, 20-HETE relaxes human bronchi under basal conditions or airways preconstricted by histamine (115). In contrast, 20-HETE increases tension in guinea pig bronchi preconstricted with histamine. Together, these results suggest species-dependent effects of this metabolite on airway smooth muscle tone.

Effects on Pulmonary Vascular Tone

20-HETE increases the diameter of isolated, pressurized human pulmonary arteries (~350 μm diameter) in a concentration-dependent and indomethacin-inhibitable manner (5). In addition, 20-HETE decreases the tone of rabbit pulmonary artery rings preconstricted with phenylephrine, and inhibition of 20-HETE formation shifts the phenylephrine concentration-response curve to the left, which is consistent with the loss of a proconstrictive effect of this metabolite on airway smooth muscle (35). On the other hand, all EET regioisomers contract isolated pulmonary arteries and rabbit pulmonary arteries.

Schwartzman and co-workers (85) have shown that 5,6-EET causes relaxation of rabbit pulmonary artery rings at concentrations as low as 40 nM. Tan and co-workers (94) observed that 5,6-EET-induced increases in perfusion pressure of isolated rabbit lungs preconstricted with the thromboxane mimetic U-46619. Similarly, Stephenson and co-workers (90, 91) noted that 5,6-EET decreased pulmonary vascular resistance in isolated, perfused dog lungs preconstricted with U-46619, an effect that was primarily attributable to reduced venous rather than arterial tone. These data suggest the position that EETs are also vasodilatory in the intact pulmonary circulation. On the other hand, all EET regioisomers contract isolated, pressurized rabbit pulmonary arteries in an endothelium- and cyclooxygenase-dependent manner (113). Furthermore, inhibition of endogenous epoxygenases in rabbit pulmonary artery rings shifts the phenylephrine concentration-response curve to the right, which is consistent with the loss of a proconstrictive metabolite. It is likely that the effect of EETs on pulmonary arteries and veins depends on variables such as species, preexisting tension or state of activation of the vessel, or on the expression of other eicosanoid-metabolizing enzymes such as the cyclooxygenases. Nevertheless, these observations do suggest that epoxygenase products may contribute to the tone of pulmonary arteries and veins.

Anti-Inflammatory Actions

Nanomolar concentrations of 11,12-EET or overexpression of CYP2J2 decreased cytokine-induced upregulation of cell adhesion molecules (including vascular adhesion molecule-1 (VCAM-1), E-selectin, and intercellular adhesion molecule-1 (ICAM-1)) in endothelial cells and inhibited leukocyte adhesion to the vascular wall (70). These effects were mediated by inhibition of nuclear factor-κB and inhibitor κB (IκB) kinase. Given the abundance of CYP2J2 in pulmonary artery endothelial cells, these data suggest that epoxygenase metabolites of arachidonic acid could play important anti-inflammatory roles in the lung, as well as in other vascular beds expressing P-450 epoxygenase isoforms.

Mitogenic Effects

EETs inhibit PGE2 formation by microvascular smooth muscle cells in a regiospecific and reversible manner (26), an effect that appears to be attributable to competitive inhibition of PGHS. This inhibitory effect of EETs was accompanied by potentiation of platelet-derived growth factor-induced smooth muscle proliferation, the net result being proproliferative effects of these metabolites. Likewise, EETs have been reported to enhance the growth of human squamous carcinoma cells in culture and reduce expression of the adhesion molecule E-cadherin (24). EETs have been observed to stimulate tubule formation in rat cerebrovascular endothelial cells (64) and to mediate epidermal growth factor-induced mitogenesis in renal epithelial cells by tyrosine kinase-dependent mechanisms (14, 15). These data support a mitogenic role for EETs in extrapulmonary tissues and raise the possibility that these eicosanoids could subserve a similar function in the lung.

Effects on Platelet Aggregation/Thrombosis

Platelet thromboxane biosynthesis can be inhibited by some but not all EET regioisomers and stereoiso- mers (28). Furthermore, arachidonic acid-induced platelet aggregation is inhibited by all EET regioiso- mers via a mechanism that is not necessarily dependent on EET-associated decreased thromboxane release (28). There are no data regarding the antiaggregatory properties of EETs in the pulmonary circulation.

Cellular Injury Due to Hypoxia-Reoxygenation

P-450-derived eicosanoids appear to protect endothelial cells from hypoxia-reoxygenation injury. Exposure of endothelial cells to hypoxia-reoxygenation resulted in appreciable cell death. Transfection of the endothelial cells with the CYP2J2 cDNA or treatment with synthetic EETs significantly attenuated hypoxia-reoxygenation-induced endothelial cell injury (B. Yang, L. Graham, J. R. Falck, J. K. Liao, and D. C. Zeldin; unpublished observations). EETs have also been shown to improve cardiac myocyte function following prolonged global ischemia in rats (103). There are currently no data on the potential role of EETs in limiting hypoxia-reoxygenation-induced cell injury in the lung.
HYPOTHESIS: CYP4A/20-HETE IS INVOLVED IN CONTROLLING THE RESPONSE OF THE PULMONARY VASCULATURE TO HYPOXIA

Despite extensive investigation, the mechanism(s) that underlie acute hypoxic pulmonary vasoconstriction is incompletely understood. Many factors including NO, prostanoids, and endothelin-1 have been proposed as possible modulators of the acute hypoxic response of the pulmonary vasculature (32, 96), but none appear to account for all of the features of hypoxic vasoconstriction. Given the abundance of CYP4A isoforms in the pulmonary vasculature (58, 114), the observation that 20-HETE dilates isolated, pressurized pulmonary arteries and relaxes pulmonary artery rings in vitro (5, 112), and the steep oxygen dependence of CYP4A catalytic activity (36, 112), we postulate that CYP4A/20-HETE is strategically positioned and well equipped to modify the response of the pulmonary vasculature to hypoxia. In support of this hypothesis, two mechanistically and structurally distinct ω-hydroxylase inhibitors 17-ODYA or DDMS increased baseline pressures of isolated blood- and buffer-perfused lungs above that of vehicle and amplified hypoxia-induced increases in perfusion pressure by approximately twofold (112). These data support the position that P-450-derived eicosanoids, like prostacyclin and NO, oppose hypoxic pulmonary vasoconstriction. However, pulmonary vasodilators are a double-edged sword under conditions of hypoxia in that they sustain perfusion to hypoventilated regions of the lung, thus worsening systemic hypoxemia (e.g., 88). In this respect, pulmonary CYP4A/20-HETE is different from prostacyclin and NO in that endogenous production of both these agents is upregulated by subacute or chronic hypoxia (16, 46, 82), whereas subacute exposure to hypoxia inhibits 20-HETE formation. Therefore, we speculate that in addition to blunting abrupt hypoxia-induced increases in pulmonary vascular tone, inhibition of 20-HETE biosynthesis under conditions of chronic hypoxia might afford a mechanism to divert blood flow away from localized areas of lung exposed to persistent hypoventilation, thus better matching ventilation and perfusion.

If these hypotheses are true, individuals with high pulmonary vascular expression/activity of CYP4A subfamily P-450s should be less susceptible to abrupt hypoxia-induced increases in pulmonary vascular tone relative to those with low CYP4A pulmonary vascular expression/activity. Similarly, individuals with high CYP4A expression should be better able (at least subacutely) to match ventilation and perfusion during conditions of localized lung injury. A better understanding of the role of CYP4A in the pulmonary vascular hypoxic response will require careful elucidation of the long-term effects of hypoxia on pulmonary CYP4A gene expression. For example, CYP4B mRNA is greatly increased in the corneal epithelium following exposure to hypoxia (60). Similar investigations of CYP4A expression, at the mRNA, protein, and enzyme activity levels, are urgently needed.

HYPOTHESIS: EETS ARE INVOLVED IN MODULATING AIRWAY SMOOTH MUSCLE TONE, LUNG INFLAMMATION, AND THE COMPOSITION OF AIRWAY LINING FLUID

Given the abundance of P-450 epoxygenases in bronchial smooth muscle and respiratory epithelium (84, 108) and the capacity of EETs to relax bronchial rings (110, 115), EETs are well equipped to serve a role in modulating airway tone. The biological imperative to maintain airway tone is sufficiently high that one would anticipate redundant mechanisms to counteract bronchospasm. Thus one would expect that the anti-bronchospastic actions of endogenous modulators of airway tone such as PGE₂ and NO might be supported by other systems. Investigations to examine the contribution of P-450 products to airway smooth muscle tone under basal conditions and under conditions associated with airway hyperresponsiveness are urgently needed. Information regarding the capacity of normal and inflamed airways to convert arachidonic acid into epoxygenase metabolites is also critical. Data from experimental animals with targeted gene disruptions should be very valuable in this regard. Together, these experiments should shed light on the potential contribution of P-450 epoxygenase metabolites in controlling bronchial tone, under both physiological and pathological conditions.

The inhibitory effects of EETs (or CYP2J2 overexpression) on endothelial cell expression of adhesion molecules, such as VCAM-1, ICAM-1, and E-selectin, and the protective effects of EETs against tumor necrosis factor α-induced mononuclear cell adhesion to the vascular wall in murine carotid arteries (70) suggests that expression EET-forming P-450s may be important in limiting lung inflammation. Given the substantial levels of CYP2J proteins in pulmonary artery endothelial cells (108) and the well-recognized role of pulmonary leukocyte sequestration in clinical and experimental lung injury (40, 87), it is possible that CYP2J products constitute an important line of defense against acute lung injury. Given the oxygen sensitivity of P-450 isoforms, it is also tempting to speculate that hypoxia-induced leukocyte sequestration (which is more evident in some species and strains than others) may be reinforced by decreased availability of epoxygenase products.

EETs play important roles in controlling renal fluid electrolyte transport and are therefore involved in regulating urinary volume composition (10, 62). Given the abundance of P-450s in the airway epithelium (84, 108) and the effects of EETs on airway epithelial Cl⁻ secretion (74, 83), it is possible that these eicosanoids regulate the volume and electrolyte composition of airway-lining fluids. Currently, there is no direct evidence to support this hypothesis; however, it is tempting to speculate that P-450-derived eicosanoids are involved in these processes in the lung as they are in the kidney.
HYPOTHESIS: ALTERATIONS IN P-450 REGULATION AND/OR FUNCTION DUE TO GENETIC POLYMORPHISM WILL BE ASSOCIATED WITH LUNG DISEASE

There are recent reports of naturally occurring mutations in the human 5-lipoxygenase gene that render transcription less effective in asthmatic patients compared with normals (44); however, a clear association of these polymorphisms with disease remains to be established. Cytochrome P-450 genes are known to be highly polymorphic, and these polymorphisms have been associated with altered drug metabolism and carcinogenesis (67). For example, polymorphisms in the CYP2C9 gene may be associated with lung cancer risk (73). Thus it is intriguing to hypothesize that alterations in P-450 expression and/or function as a result of genetic polymorphism might be associated with lung disease. Single nucleotide polymorphisms within the coding and/or promoter regions of P-450 genes could account for altered capacities of individuals to biosynthesize EETs and 20-HETE. Indeed, recent data indicate that the human CYP2J2 gene is polymorphic and that several of the coding single nucleotide polymorphisms result in altered P-450 catalytic function (51). If the expression of CYP2J genes in the airway has an overall beneficial effect on airway smooth muscle tone, persons with diminished capacity to synthesize EETs might be more susceptible to bronchospasm. Similarly, because 11,12-EET appears to repress tandem kB sites in the VCAM-1 promoter (and decrease adhesion molecule-mediated leukocyte adhesion to the vascular wall), it is possible that mutations resulting in either decreased EET synthesis or decreased interaction of EETs with kB cis-acting elements of the VCAM-1 promoter may enhance host susceptibility to lung inflammation and/or acute lung injury. Polymorphisms in the CYP4A genes could contribute to enhanced susceptibility to hypoxia-induced pulmonary hypertension or impaired ability to match ventilation and perfusion under pathophysiological conditions. Identification of genetic polymorphisms and their phenotypic characteristics will almost certainly enhance our understanding of the role of P-450s and their eicosanoid products in the modulation of critical lung functions.

HYPOTHESIS: P-450-DERIVED EICOSANOIDS WORK THROUGH CELL SURFACE RECEPTORS

It is now well established that prostaglandins and leukotrienes mediate their actions by interaction with specific cell surface receptors (6, 18, 20, 65). The cloning and characterization of prostaglandin and leukotriene receptors have revolutionized the eicosanoid field and have led to the development of specific agonists and antagonists that have been used experimentally and clinically (21). Moreover, elucidation of their intracellular signaling mechanisms has led to the identification of a variety of downstream targets for modulating these eicosanoid pathways. As mentioned, there is now evidence for surface-binding receptor sites for 14R,15S-EET in guinea pig mononuclear cells and U-937 monocyte cells (99, 100). Similarly, functional data from isolated, pressurized renal arteries using 20-HETE analogs suggest receptor-dependent vasoactivity in the kidney (2). There is no information regarding the nature, location, or existence of EET and 20-HETE receptors in the lung. At the time of this writing, none of these putative receptors have been cloned or characterized; however, it is reasonable to postulate that specific cell surface receptors exist for the EETs and HETEs, as they do for the prostaglandins and leukotrienes.

TOOLS TO EXPLORE THE FUNCTIONAL SIGNIFICANCE OF P-450 EXPRESSION

Transgenic Mouse Models

Targeted disruption of the PGHS and lipoxygenase genes have led to improved understanding of the role of these eicosanoid-metabolizing enzymes in lung health and disease (30, 45). Mouse lines with targeted disruptions in several P-450 genes have been available for the last several years. Studies with these mice have led to a better understanding of the role that P-450 genes play in development and xenobiotic metabolism (63, 66). For example, disruption of the CYP1A2 gene resulted in neonatal death due to severe respiratory distress (77). The penetrance of the lung phenotype was incomplete, and the limited number of mice surviving to adulthood were healthy, fertile, and lacked lung abnormalities. In contrast, a second CYP1A2-/- mouse line produced fertile and histologically normal mice (54). These apparently conflicting studies reinforce the fact that the size of the deleted gene fragment, the mouse genetic background, and other factors may impact the observed phenotype. The CYP2E1 gene has also been disrupted; however, no overt phenotypic abnormalities other than altered acetaminophen hepatotoxicity were reported in the homozygous null mice. These data raise the possibility that disruption of P-450 genes may contribute to pulmonary phenotypic abnormalities and suggest the utility of further applications of this approach. Recently developed inducible or conditional knockout systems (e.g., Cre recombinase, reverse tet system) should allow study of the effects of “embryonic lethal” genes on lung function in adult animals (66). The availability of lung-specific promoters that drive expression of transgenes in airway epithelial cells (CC10 promoter) and alveolar epithelial cells (SP-C promoter) (39, 80, 98) should also facilitate future studies on the effects of overexpression of P-450s in the lung.

Congenic Strains of Mice and Rats

Congenic strains of experimental animals are produced by backcrossing progeny for a number of generations to allow fixing of the genetic background with the exception of the genetic loci of interest. For example, Jiang and colleagues (49) utilized congeneric strains of rats to demonstrate that transfer of a salt-resistant renin allele to a salt-sensitive strain raises plasma renin activity and worsens hypertension and renal
disease. Similar comparisons should yield critical structural and functional information regarding the role of eicosanoid-metabolizing P-450s in the lung.

**Structurally Related 20-HETE and EET Agonists/Antagonists**

Recently, a number of structurally related chemicals that function as 20-HETE agonists or antagonists with respect to the vasoconstrictor response of interlobular arteries have been developed and tested (2). For example, 20-hydroxyeicosa-6Z,15Z-dienoic acid completely blocks 20-HETE-dependent constriction of renal arteries. These studies not only help to identify the structural requirements for 20-HETE biological activity, but also they afford a unique opportunity to activate or inhibit the putative 20-HETE receptor in experimental models. Similarly, Falck and co-workers (14) recently developed several stable EET analogs and showed that they retain biological potency in vitro. P-450 eicosanoid agonists and antagonists such as these should allow investigators to study the role of 20-HETE and EETs in models such as the isolated, perfused lung and in vivo animal models of bronchospasm, lung inflammation, and lung injury.

**Specific P-450 Inhibitors**

Historically, P-450 inhibitors (e.g., 5,8,11,14-eicosa-

tetraynoic acid, SKF-525A, and 17-ODYA) have been plagued by lack of isoenzyme specificity and by nonspecific effects on other eicosanoid-metabolizing pathways. A new generation of P-450 inhibitors have emerged that have isoenzyme-specific compounds. For example, 6-(20-

-propargyloxyphenyl)hexanoic acid inhibits conversion of arachidonic acid into EETs in concentrations more than 10- to 100-fold lower than those required to inhibit formation of HETEs (68). Administration of a single dose of 1-aminobenztriazole blocks formation of 20-HETE with no significant effect on the synthesis of epoxide products in rat renal cortex. Importantly, 1-aminobenztriazole has been shown to lower blood pressure in spontaneously hypertensive rats (92). Use of these specific P-450 inhibitors in vitro in cell culture systems and in vivo in animal models of disease will undoubtedly help investigators to elucidate the functional roles of these enzymes in lung physiology and pathology.

**Inhibitory Antibodies, Antisense Oligonucleotides, and Hammerhead Ribozymes**

Traditionally, inhibition of P-450 enzyme activity has been accomplished with isoenzyme-specific antibodies (10, 11, 109, 110). With sequences for a growing number of P-450 isofoms now available, some groups have successfully used antisense oligonucleotides to inhibit P-450-derived eicosanoid formation and to elucidate the functions of individual P-450 isofoms in a given tissue or cell system. For example, Schwartzman and colleagues (95) demonstrated that rat renal 20-HETE formation can be effectively blocked by infusion of CYP4A1 antisense oligonucleotides. Similarly, Fissl-

thaler and co-workers (27) used antisense to CYP2C8/9 to inhibit EDHF-mediated relaxation of porcine coronary arteries. The recent development of catalytically active hammerhead ribozymes offers an effective alternative to antisense oligonucleotides for specific inactivation of gene expression (12).

**Adenovirus and Adeno-Associated Viral Delivery of P-450 cDNAs**

Recent advances in gene delivery using adenovirus and adeno-associated viral vectors have facilitated studies on the effects of overexpression of specific genes both in vitro and in vivo (34, 102). These methods have recently been used by investigators to replace the defective CFTR gene in cystic fibrosis (53, 116). Similar methods could be used to deliver P-450 genes to the airway or pulmonary vasculature.

**CONCLUSIONS AND PREDICTIONS**

Judicious application of the above tools should allow investigators to examine the functional role of P-450s in lung health of disease. However, each of these approaches has limitations. For example, the extensive homology between P-450 isoforms within a given sub-family makes utilization of antisense oligonucleotides potentially problematic. Compensatory upregulation or downregulation of related isoforms in knockout or transgenic strains may complicate efforts to correlate genotypes and observed phenotypes. Therefore, combinations of methodologies will likely be required to elucidate the endogenous functions of P-450s in the lung. Additionally, it is essential that investigators document altered P-450 expression and function with use of these techniques. Despite these caveats, we predict that experiments using these and other novel methodologies will soon identify critical roles for and circumstances of participation of P-450 isoforms in pulmonary inflammation, control of pulmonary vascular and bronchomotor tone, and lung epithelial ion transport. Furthermore, knowledge of the relationships between altered pulmonary P-450 expression and lung pathophysiology should suggest specific targeted interventions that will expand therapeutic options for individuals with pulmonary disease. Not all of these hypothesized functions may prove to be correct, but we now have the means and the incentive (if not imperative) to put them to the test.

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