Excess nitric oxide decreases cytochrome P-450 2J4 content and P-450-dependent arachidonic acid metabolism in lungs of rats with acute pneumonia

Asma Yaghi, John R. Bend, Christopher D. Webb, Darryl C. Zeldin, Sean Weicker, Sanjay Mehta, and David G. McCormack.

Excess nitric oxide decreases cytochrome P-450 2J4 content and P-450-dependent arachidonic acid metabolism in lungs of rats with acute pneumonia. Am J Physiol Lung Cell Mol Physiol 286: L1260–L1267, 2004. First published February 6, 2004; 10.1152/ajplung.00273.2003.—Recently, we demonstrated that pulmonary CYP2J4 content, a prominent source of EETs and HETEs formation in rat lungs, is reduced in pneumonia. Therefore, the purpose of this study was to determine the role of iNOS-derived NO in reduced pulmonary CYP2J4 protein content and decreased CYP metabolites in pneumonia. Rats were randomized to control, control plus 1400W (iNOS inhibitor), pneumonia, and pneumonia plus 1400W groups. Pseudomonas organisms were injected into lungs of pneumonia rats. At 40 h after surgery, rats were treated with either saline or 1400W for 4 h before death. Venous plasma samples were obtained for measuring nitrites/nitrates (NOx). There was no significant effect of 1400W on blood pressure measured in control or pneumonia rats, whereas 1400W reduced the elevated plasma NOx levels in pneumonia rats by half. CYP primary metabolites of AA formed at significantly lower rates in pulmonary microsomes from pneumonia rats compared with control rats. Treatment of pneumonia rats with 1400W resulted in a significant increase in the rate of formation of pulmonary EETs and ω-terminal HETEs compared with untreated pneumonia rats. The reduction in CYP2J4 protein content in pneumonia lung microsomes was also partially prevented by 1400W. Therefore, excess NO from iNOS decreases the pulmonary production of EETs and ω-HETEs in acute pneumonia. Inhibition of iNOS restores CYP2J4 protein content and CYP activity in acute pneumonia, indicating an important NO-CYP interaction in pulmonary responses to infection. We speculate CYP2J4 and its AA metabolites are involved in the modulations of pulmonary function in health and disease.

inflammation; CYP2J4 protein content; CYP450 activity assays; epoxyeicosatrienoic acids and ω-terminal hydroxyeicosatetraenoic acids; 1400W

EVIDENCE FROM THE LITERATURE indicates that arachidonic acid (AA) metabolites and nitric oxide (NO) contribute to the effects of sepsis and inflammation (6, 28, 29, 38). However, whether a link exists between the two pathways (AA cascade and NO synthase) in acute pneumonia is not clear. We have previously demonstrated depressed contractility of small pulmonary artery (PA) rings dissected from lungs of rats with an acute and localized Pseudomonas pneumonia (44, 45). With the use of inhibitors of cyclooxygenase enzymes and the inducible isoform of NO synthase (iNOS), we ruled out the involvement of relaxant prostaglandins (PGL2 and PGE2) and established the involvement of excess NO (from iNOS) in this depressed contractility (44, 45). In addition, we demonstrated that total prostaglandin levels, and more specifically PGE2 and 6-keto-PGF1α, a byproduct of prostacyclin, are not different in pneumonia compared with control lungs. However, the rate of production of 20-hydroxyeicosatetraenoic acid (20-HETE) and epoxyeicosatrienoic acids (EETs), cytochrome P-450 (CYP) metabolites of AA, is depressed in lungs from pneumonia rats (45). In addition, some of the CYP monooxygenase metabolic products of AA have vasoactive effects and could play a central role in the regulation of vascular tone responses (27, 36, 37). We recently demonstrated that EETs and 20-HETE are potent vasoconstrictors of small PA rings from control and pneumonia rat lungs (45). Furthermore, this potency was attenuated in PA rings from pneumonia lungs compared with control lungs, indicating a potential role for these metabolites in the contractile responses of both normal pulmonary blood vessels and in the setting of pneumonia.

CYP metabolites of AA are produced by lung microsomes of all studied species and exist endogenously in lung tissue (10, 15). Whether epoxygenation products or ω-hydroxylation products predominate depends on the specific CYP isoforms present in lung. CYP2J3 and CYP2B isozymes produce mainly EETs (16, 46, 47). Prominent expression of CYP2J3 isoforms in vascular smooth muscle and vascular endothelium has been demonstrated in human and rat lung (46). With the use of peptide-based antibodies to members of the CYP2J3 subfamily, we recently demonstrated that rat lung contains a protein with immunologic characteristics identical to CYP2J4 and that microsomal content of this isozyme, but not CYP2B1, is reduced following pneumonia (43).

Studies with rat hepatocytes demonstrate that NO inhibits CYP catalytic activity in vitro in an isozyme-selective manner (3, 40). Recently, in renal tissues, Oyekan et al. (23) demonstrated an inhibitory role for NO on activity and expression of renal CYP4A. In hepatic tissues, evidence of a decline in CYP protein in models of inflammation and infection has also been reported (19, 35). However, whether or not NO produced by iNOS in models of inflammation participates in the reduction of CYP catalytic activities as well as protein and mRNA expression remains controversial and could be dependent on...
the model studied, the tissues investigated, and the isozyme(s) involved (12, 22, 32, 34). The acute pneumonia model in the rat is characterized by increased lung iNOS activity and elevated plasma nitrite/nitrate (NOx) levels. Therefore, we proposed that excess NO (from iNOS) contributes to the CYP metabolic and protein (CYP2J4) changes observed in acute Pseudomonas pneumonia. To test this hypothesis, we used N-[3-(aminomethyl)benzyl]acetamidine dihydrochloride (1400W), a novel and highly selective inhibitor of iNOS activity (4, 5). The purpose of this study was twofold: 1) to determine whether inhibition of iNOS (and excess NO) reverses the decline in the pulmonary production of EETs and α-terminal HETEs observed in pneumonia and 2) to determine whether inhibition of NO synthesis (through iNOS inhibition) could restore CYP2J4 protein content in pneumonia lungs. Here, we demonstrate that in rats with acute pneumonia, inhibition of iNOS with 1400W increased pulmonary CYP activity and CYP2J4 protein content in lung compared with untreated pneumonia rats.

MATERIALS AND METHODS

All animals used in this study were cared for following the principles and guidelines of the Canadian Council on Animal Care and were supervised by a veterinarian. In addition, the ethics review committee at the University of Western Ontario (London, ON, Canada) approved all protocols.

Acute pneumonia model. The acute pneumonia rats were prepared as described previously (44) with the addition of a carotid line to measure blood pressure (BP) in rats from all groups. Briefly, male Sprague-Dawley rats (275–350 g) were randomized to control (n = 6), control plus 1400W (n = 6), pneumonia (n = 8), and pneumonia plus 1400W (n = 11) groups. Animals in all groups were anesthetized with halothane, and a jugular venous line was placed for fluid administration. Animals in the pneumonia groups were injected intratracheally with Pseudomonas aeruginosa, 0.15 ml of saline containing 3 × 10⁸ colony forming units/ml, through a tracheostomy. Within 36 h, this instillation of bacteria produced an acute localized pneumonia (left affected) lobes from pneumonia rats compared with control rats and pneumonia (left affected) lobes from pneumonia rats.

Measurement of NO metabolites in plasma. Plasma samples were obtained at 40, 40.5, 41, 42, and 44 h postsurgery (the time of death). Note that at 40 h, the plasma samples were obtained just before the start of the saline bolus (control and pneumonia) or 1400W bolus + infusion (control + 1400W and pneumonia + 1400W). *P < 0.05 compared with control (saline) and control + 1400W; #P < 0.05 compared with saline-treated pneumonia.

Fig. 1. Effect of inducible isofrom of nitric oxide synthase (iNOS) inhibition with 1400W on plasma nitrites/nitrates (NOx) in rats with pneumonia. Plasma NOx concentrations from control (n = 4), control + 1400W (n = 5), pneumonia (n = 5), and pneumonia +1400W (n = 10) rats are shown. Samples were obtained at 40, 40.5, 41, 42, and 44 h postsurgery (the time of death). Note that at 40 h, the plasma samples were obtained just before the start of the saline bolus (control and pneumonia) or 1400W bolus + infusion (control + 1400W and pneumonia +1400W). *P < 0.05 compared with control (saline) and control +1400W; #P < 0.05 compared with saline-treated pneumonia.
CYP2J6pep1) were similarly prepared in rabbits. This antibody immunoreacts with mouse CYP2J6 and rat CYP2J4 but does not cross-react with other CYP2J isoforms. Polyclonal antibodies against the human CYP2J2 polypeptide RESMPYTVHEVQRMGNIPQN (anti-CYP2J2pep3) were raised in rabbits and cross-react with all known CYP2J subfamily P-450 isozymes. None of these antibodies is known to cross-react with non-CYP2J subfamily P-450 isozymes. Preimmune serum, collected from the rabbits before immunization, did not cross-react with recombinant CYP2J isoforms (data not shown).

SDS-PAGE and Western blotting for CYP protein. Lung microsomal proteins were electrophoresed on SDS-10% polyacrylamide gels, and the resolved proteins were transferred electrophoretically onto PVDF (polyvinylidene difluoride) membranes (Amersham Canada, Oakville, ON). Membranes were immunoblotted using anti-CYP2J9pep2, anti-CYP2J6pep1, or anti-CYP2J2pep3. All the CYP2J antibodies were used at a 1:1,000 dilution. Bound antibodies were detected using goat anti-rabbit IgG conjugated with horseradish peroxidase (dilution 1:2,000; Promega, Madison, WI) and the enhanced chemiluminescence Western blotting detection system (ECL Plus, Amersham Canada). Relative molecular weights of proteins were determined by running a prestained protein marker (Promega) on all gels. In addition, microsomes from Sf9 cells expressing recombinant CYP2J3 were used as a standard. Films were scanned, and the intensities of the bands were quantified via photodensitometry. Multianalyst 1.1 (Bio-Rad Laboratories, Hercules, CA) was used for measuring optical densities (OD). Note that OD × mm² = OD multiplied by area in millimeters squared.

Chemicals. All salts for Krebs solution were purchased from BDH Chemicals (Toronto, ON, Canada). [14C]AA (NEC-661, purity >99%) was purchased from Mandel Scientific (Guelph, ON, Canada). 1400W dihydrochloride was purchased from Cayman Chemicals (Ann Arbor, MI) and dissolved in saline before being administered to rats. Potassium phosphate monobasic was purchased from Fisher Scientific (Mississauga, ON, Canada). Cytoscint Environmentally Safe scintillation fluid and Coomassie brilliant blue G250 were purchased from ICN Biomedical (Mississauga). Reagents required for SDS-PAGE electrophoresis, transfer, and Western blotting of proteins were purchased from Amersham Canada. Blotto in Tris-buffered saline (Pierce, Rockford, IL) was used for blocking the PVDF membranes. All other reagents were purchased from Sigma-Aldrich Canada (Mississauga).

**Fig. 2.** Effect of iNOS inhibition with 1400W on blood pressure. Blood pressure in control (n = 6), control + 1400W (n = 5), pneumonia (n = 6), and pneumonia + 1400W (n = 11) rats was measured at time of death, 44 h postsurgery. There was no significant effect of 1400W on blood pressure measured in control or pneumonia rats.

**Fig. 3.** Hematoxylin and eosin staining of sections of lung tissues from control (A), control + 1400W (B), pneumonia (C), and pneumonia + 1400W (D) rats. Magnification ×50. Examination of lung sections from control (A) and control + 1400W (B) rats showed no inflammatory cells. Lung specimens from pneumonia (C) and pneumonia + 1400W (D) rats showed accumulation of inflammatory cells in the lung. The histology was determined in a minimum of 5 rats per group.
Data analysis. All figures were plotted using Prism (GraphPad Software, San Diego, CA) and analyzed using Prism or Graphpad Instat (GraphPad Software). All results are expressed as means ± SE of the mean of n values, where n = number of rats. Results were compared using ANOVA followed by Bonferroni posttests. A value of $P < 0.05$ was considered significant.

RESULTS

Effect of iNOS inhibition on plasma NOx. Plasma NOx was significantly elevated in pneumonia compared with control rats (Fig. 1). Treatment of pneumonia rats with 1400W significantly reduced the plasma NOx concentration 2 h after the start of the 1400W bolus plus infusion (42 h, Fig. 1). In addition, the plasma NOx concentration in pneumonia rats was reduced to near that which was observed in control rats 4 h after the start of the 1400W bolus plus infusion (44 h, Fig. 1). However, 1400W did not alter NOx levels in control rats.

Effect of iNOS inhibition on BP. BP measurements from control, pneumonia, control plus 1400W, and pneumonia plus 1400W rats were similar and did not differ among groups (Fig. 2). Data in Fig. 2 show BP measurements taken at 44 h postsurgery, right before death. Similar values were obtained at 24 h postsurgery and when measured serially between 40 and 44 h (data not shown).

Effect of iNOS inhibition on accumulation of inflammatory cells in the lungs. Histology was done on lungs from the four groups of rats (control, control + 1400W, pneumonia, and pneumonia + 1400W). As shown in Fig. 3, 1400W did not affect the accumulation of inflammatory cells in the lungs of rats with pneumonia.

Effect of iNOS inhibition on pulmonary CYP metabolites of AA. CYP metabolites of AA were analyzed in lung microsomes from control, pneumonia, control plus 1400W, and pneumonia plus 1400W rats. HPLC chromatograms of the [14C]AA metabolites confirmed that two major classes of CYP primary metabolites, EETs and ω-terminal HETEs, were formed at significantly lower rates in lung microsomes from pneumonia rats compared with microsomes from control rats and that treatment of pneumonia rats with 1400W resulted in increased rates of formation of EETs and ω-HETEs compared with untreated rats with pneumonia (Fig. 4). The rates of formation of EETs in lung microsomes from control and pneumonia rats treated with 1400W or vehicle (saline) are shown in Fig. 5A. Similarly, the rates of formation of ω-terminal HETEs in these lung microsomes are shown in Fig. 5B. Specific activities of the CYP-dependent formation of EETs and ω-HETEs were depressed in pneumonia lungs compared with controls. Treat-

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Fig. 4. Representative HPLC chromatograms of the NADPH-dependent [14C]arachidonic acid (AA) metabolites formed by pulmonary microsomes from control (A), control + 1400W (B), pneumonia (C), or pneumonia + 1400W (D) rat lungs. Note that the peaks for ω-terminal HETE and epoxyeicosatrienoic acid (EET) metabolites that were decreased in pneumonia rats were partially restored in pulmonary microsomes from pneumonia rats treated with 1400W. cpm, Counts per minute.
ment of pneumonia rats with 1400W resulted in a significant decrease in the rate of formation of ω-HEtEs (Fig. 5A) and ω-HEtEs (Fig. 5B) compared with untreated (saline) pneumonia rats. However, these activities were slightly lower than those in lung microsomes of control rats.

**DISCUSSION**

In this study, we verified that EETs and ω-terminal HEtEs are formed at significantly lower rates in microsomes from lungs of pneumonia rats compared with those of control rats (45) and that CYP2J4 protein content is reduced in lungs following pneumonia (43). As expected, administration of 1400W was shown to inhibit plasma NOx accumulation in rats with pneumonia (Fig. 1). iNOS inhibition in pneumonia increased the rate of formation of pulmonary EETs and ω-HEtEs compared with untreated (saline) pneumonia rats (Fig. 5), indicating that iNOS-derived NO attenuates the production of these CYP metabolites of AA in pneumonia. In addition, inhibition of excess NO from iNOS partially restored CYP2J4 protein content in pneumonia lung microsomes (Figs. 6 and 7). Note that 1400W blocked the elevation of plasma NOx levels in rats with pneumonia without any effect on NOx levels of control rats (Fig. 1) or any effect on the accumulation of control rats with 1400W did not significantly modify the rates of formation of ω-HEtEs or EETs. However, treatment of pneumonia rats with 1400W resulted in a significant increase in the rate of formation of EETs (Fig. 5A) and ω-HEtEs (Fig. 5B) compared with untreated (saline) pneumonia rats. However, these activities were slightly lower than those in lung microsomes of control rats.

**Effect of iNOS inhibition on CYP2J4 content.** Western blotting for CYP2J4 protein revealed a prominent 55-kDa band with anti-CYP2J2pep3, which immunoreacts with all CYP2J isoforms, and anti-CYP2J6pep1, which immunoreacts only with CYP2J4, that was similar in intensity in lung microsomes from control and control plus 1400W rats (Fig. 6A). In comparison, the CYP2J4 band that was reduced in pneumonia compared with control lung microsomes was partially restored in lung microsomes from pneumonia rats treated with 1400W (Fig. 6A). Reprobing the same blots with anti-CYP2J9pep2, which immunoreacts with CYP2J3, revealed a band only for the 2J3 standard (data not shown). Inhibition of iNOS with 1400W revealed a CYP2J4 band when 20 or 40 μg of pulmonary microsomal protein from pneumonia rats were electroblotted (Fig. 6B). Analysis of the CYP2J4 band densities indicated that this protein was significantly restored when loading 20 or 40 μg of pulmonary microsomal protein from pneumonia plus 1400W rats (Fig. 7).

![Image](http://www.ajplung.org/)

**Fig. 5.** Specific activities for formation of EETs (A) and ω-terminal HEtEs (B) in lung microsomes from control (n = 6), control+1400W (n = 6), pneumonia (n = 8), and pneumonia+1400W (n = 11) rats. Note the effect of inhibition of iNOS with 1400W on pulmonary production of cytochrome P-450 (CYP) metabolites in vitro in pneumonia *P < 0.05 compared with control (saline) and control+1400W; #P < 0.05 compared with saline-treated pneumonia.

**Fig. 6.** Representative Western blots of pulmonary microsomes (20 μg of protein each for A and 20 and 40 μg of protein in B) from control, control+1400W, pneumonia, and pneumonia+1400W rats. Polyvinylidene difluoride membranes were immunoblotted with different CYP2J antibodies, and bands were identified using a molecular weight marker (not shown) and CYP2J3 standard. Blots in A are identical; microsomes (20 μg of protein) from same lung samples were loaded on 2 gels and electrophoresed in parallel. Each was then probed with the antibodies as indicated. Stripping and reprobing the top blot with anti-CYP2J6pep1 resulted in an identical blot to what is shown for the bottom blot and visa versa for the top blot when stripped and reprobed with anti-CYP2J2pep3. Naïve lung: lung microsomal protein from untreated animals, whereas this band was virtually absent from pneumonia lung microsomes.
of inflammatory cells in the lungs of rats with pneumonia (Fig. 3). This demonstrates that the decline in CYP2J4 protein and activity was not influenced by influx of inflammatory cells or the accumulation of protein-rich exudates in lungs from rats with pneumonia. It is known that EETs and 20-HETEs are potent constrictors of small pulmonary arteries in the rat (45) and that CYP2J isozymes are a prominent source of EETs and 20-HETEs in rat lungs (46). Consequently, decreased CYP2J4 content and the resultant attenuated production of EETs and 20-HETEs in pneumonia lung may contribute to the reduced pulmonary vascular contractility observed in pneumonia. Thus EETs, ω-terminal HETEs, and NO, formed subsequent to induction of iNOS, are likely modulators of contractility of arteries in the pulmonary circulation both in health and disease.

NO is synthesized by NOS from the terminal guanidino group of l-arginine (8). A family of three genes has now been identified that encodes for NOS. Types I and III are the calcium-dependent isofoms, also known as constitutive NOS, first identified in neurons and endothelial cells, respectively (30). The physiological production of NO in endothelial cells by endothelial NOS (eNOS) is important for BP regulation and blood flow distribution (21). Type II NOS (iNOS) is the calcium-independent isofom, and its expression is induced by inflammatory mediators, such as cytokines and endotoxins, in a variety of tissues and cells, including the vascular endothelium, vascular smooth muscle cells, and macrophages (1, 7, 9). We have previously demonstrated that the acute pneumonia rat model is characterized by increased iNOS activity and increased NOx concentrations in lung homogenates (45). Here, we demonstrate that plasma NOx concentrations are also elevated in pneumonia rats. Administration of 1400W, a highly selective iNOS inhibitor (4, 5), reduced plasma NOx accumulation in pneumonia, confirming in vivo inhibition of iNOS in this pneumonia model of the rat. In addition, intravenous administration of 1400W did not have any effect on BP in control rats, consistent with no inhibition of eNOS activity. This selective in vivo effect of 1400W on iNOS activity has been demonstrated previously in other rat models of inflammation, where there is increased production of NO after iNOS induction (17, 24). 1400W attenuated LPS-induced microvascular injury in the rat colon (17) and prevented ischemic brain injury in rats (24). In LPS-treated rats, 1400W markedly reduced iNOS activity in the lungs with >90% inhibition obtained at 20 mg/kg 1 h after treatment (24).

Most previous reports of NO-CYP interactions concern liver. Various CYP isozymes can be suppressed or induced depending on the model studied, the stimulus used (e.g., LPS, particulate irritants, or infection), the tissues investigated, and the cytokines released (11, 12, 33). In rats treated with LPS, NO is one of the mediators of the inhibition of CYP2C11, 3A2, 1A2, and 2B1/2 isozymes in rat liver (11), and a systemic infection with Chlamydia trachomatis resulted in an NO-dependent diminished CYP1A- and CYP2B-mediated metabolism in the liver of the mice (12). Administration of irritants in rats was found to suppress hepatic CYP2C11 mRNA and protein and to induce CYP4A1, 4A2, and 4A3 mRNA expression while having no significant effect on CYP2E1 or 3A2. In comparison, CYP4A2, 4A3, and 2E1 mRNAs were all induced in the kidney of irritant- and LPS-treated rats, indicating that different inflammatory stimuli affect individual CYP isozymes differentially (33).

Endotoxic shock, which is associated with induction of iNOS and release of large amounts of NO, results in isozyme-selective inhibition of hepatic CYP-dependent metabolism (20). Acute inflammatory reactions in humans and animals diminish the amount and activity of hepatic CYP enzymes. Many studies involving treatment of whole animals with LPS, or incubation of human or rat hepatocytes with LPS or cytokines, have implicated NO as a mediator of the decreases seen in CYP metabolic activities (2, 13). NO donors and NO have also been shown to inhibit hepatic CYP activity in vitro (14, 40) and in vivo (3). NO inhibits CYP activity due to binding of NO to ferrous or ferric iron of the catalytic heme moiety (40). NO can also modulate CYP activity by diminishing the mRNA expression of selected isozymes (11, 40), and NOS inhibitors are capable of reversing the decreases in activity, protein, and mRNA of some CYP isozymes (3A2, 2C11, 1A2, and 2B1/2) in rat liver (11, 35).

NO-CYP interactions have also been demonstrated in the kidney (22). Specifically, NO inhibits renal CYP4A4 expression and activity, thus modulating renal function (23). Our rat Pseudomonas pneumonia model is an acute localized infection, characterized by increased production of NO by iNOS and depressed formation of pulmonary EETs and ω-terminal HETEs in lungs. iNOS inhibition in pneumonia increased the rate of formation of pulmonary EETs and ω-HETEs compared with untreated pneumonia rats (Fig. 5), indicating that iNOS-
derived NO attenuates pulmonary CYP metabolic activity in pneumonia.

Evidence from the literature supports abundant expression of a number of CYP monooxygenases in the lung (16, 47). Recently, molecular and immunological evidence showed that CYP2J isozymes, a prominent source of EETs and o-HETEs, are highly expressed in both human and rat lung and that expression is localized to airway epithelial cells, vascular and bronchial smooth muscle cells, vascular endothelium, and alveolar macrophages (31, 46). In our acute pneumonia model, we have previously demonstrated that CYP2J4 content is reduced following pneumonia (43). Here, we provide evidence that iNOS-derived NO either attenuates the expression or accelerates the proteolysis of CYP2J4 protein in acute pneumonia. Thus treatment with 1400W 4 h before death partially restored the 55-kDa CYP2J4 band in pneumonia compared with untreated pneumonia rats, without any effect on pulmonary CYP2J4 content in controls (Fig. 6).

CYP2J proteins are induced by pyrazole in mouse tissues, including lung (42), but little is known about the regulation of CYP2J in inflammatory conditions. In human and rat tissues, discordance between CYP2J mRNA and protein levels has been observed (31). As mentioned above, isozyme-selective suppression of CYP mRNAs, caused by cytokines and other inflammatory stimuli, has been described in models of inflammation for hepatic CYP (19). However, much less is known about pulmonary CYP isozymes in models of inflammation. In addition, CYP protein turnover rates are heterogeneous and might involve ubiquitination and/or proteasome-mediated degradation depending on the CYP isozyme involved (26). In rat hepatocytes cultured on Matrigel, participation of NO in the downregulation of CYP2B1 was dependent on the concentration of LPS used, with a rapid NO-dependent suppression of the protein, but not mRNA, occurring at high concentrations of LPS (3). It is possible that other CYP enzymes, such as CYP2J4, could be regulated in a similar manner by excess NO in the lung. We previously demonstrated that there was no decrease of CYP2B1/2 content in lungs of pneumonia rats (43), therefore indicating isozyme-selective modulation of pulmonary CYP in pneumonia. In our rat model of pneumonia, the marked decrease of pulmonary CYP2J4 protein content by excess NO from iNOS could be due to a decline in transcription or translation of this enzyme, or due to increased degradation of the protein caused by the proteasome degradation system. Earlier studies reported that NO-mediated decreases in CYP protein expression were observed as early as 6 h post-LPS treatment in cultured rat hepatocytes (3) and 12 h in rats treated with high dose of LPS (20). Intraportal administration of NOS inhibitors (aminoguanidine, Nω-monomethyl-L-arginine, Nω-nitro-L-arginine methyl ester) to rats, after they received LPS, was capable of reversing the decreases in CYP activity and content in hepatic microsomes (11, 20). In our study, the change in CYP2J4 protein occurred very quickly (in 4 h) compared with other studies, but we used intravenous administration of 1400W, which is also a more selective blocker of iNOS. Because of the short duration of treatment with 1400W (bolus + 4-h infusion), we speculate that a decrease in protein rather than mRNA and protein occurred. Further studies are needed to test whether the quick recovery (4 h) of CYP2J4 protein in lung microsomes from 1400W-treated pneumonia rats is due to modulation of protein degradation or transcription; however, the former seems more likely.

Most previous studies investigating the role of CYP metabolites of AA in inflammation concentrated on liver, due to its high CYP content and the diversity of isozymes localized there, and often utilized endotoxin models of inflammation rather than an infectious model. The novel data presented in this study provide evidence for a role for NO, produced by iNOS, in the attenuation of pulmonary CYP catalytic activity and CYP2J4 protein content in pneumonia. This NO-CYP interaction is significant, as it indicates an important role for NO, EETs, and o-HETEs in lung physiology and pathophysiology.

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