Modulation of Pulmonary Leukotriene B₄ Production by Cyclooxygenase-2 Inhibitors and Lipopolysaccharide

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ABSTRACT

Purpose: Emerging data continue to link carcinogenesis to inflammatory events involving the eicosanoid metabolic pathways. We therefore evaluated the effects of cyclooxygenase (COX)-2 inhibition on leukotriene (LT) B₄ synthesis in the lungs of active smokers, as part of a pilot lung cancer chemoprevention study with celecoxib (Celebrex), an oral COX-2 inhibitor.

Experimental Design: Bronchoalveolar lavage was performed before celecoxib treatment and after 1 month of celecoxib treatment to recover alveolar macrophages (AMs) and lining fluid for study. After harvest, AMs were immediately stimulated in vitro with the calcium ionophore A23187. AMs obtained from smokers before treatment and from ex-smoker control subjects were also cultured overnight with SC58236, a selective COX-2 inhibitor, with or without lipopolysaccharide stimulation.

Results: Treatment with oral celecoxib only modestly increased LT₄ levels in bronchoalveolar lavage, without increasing the mRNA transcription of 5-lipoxygenase (5-LOX) or 5-LOX–activating protein in AMs, whereas the acute calcium ionophore-stimulated LT₄ production from smokers’ AMs was markedly increased by 10.6-fold. In addition, smokers’ AMs were twice as responsive in producing LT₄ when exposed to lipopolysaccharide compared with ex-smokers’ AMs. Concomitant COX-2 inhibition with SC58236, however, did not significantly impact these changes, whereas the 5-LOX inhibitor Zileuton blocked the generation of LT₄ in a dose-responsive manner. Finally, cycloheximide increased the production of LT₄ under all conditions, suggesting a shunting phenomenon and/or the presence of pathway inhibitors.

Conclusions: Our findings suggest that whereas oral celecoxib is capable of modulating LT₄ production in the lung microenvironment, under physiologic conditions, this effect is probably not functionally significant.

INTRODUCTION

Ample preclinical data suggest that the cyclooxygenase (COX)-2/prostaglandin E₂ (PGE₂) signaling pathway plays an essential role in conferring the malignant phenotype in non–small-cell lung cancer (1–8). Whereas much attention has been focused on nonsteroidal anti-inflammatory drugs that target this pathway, the alternate pathways responsible for the synthesis of other proinflammatory eicosanoids, such as leukotriene (LT) B₄, and the consequences of COX-2 inhibition on LT₄ production remain largely unexplored.

Eicosanoids are biosynthesized from arachidonic acid (AA) released from membrane phospholipids via the action of phospholipase A₂. AA is then converted to prostaglandins by COXs and to LTs by lipoxygenases [LOXs (9, 10)]. LTs are eicosanoids generated through the 5-LOX pathways. The enzymatic activity of 5-LOX requires the presence of the 5-LOX–activating protein (FLAP) in intact cells (11). The final and biologically active metabolites of the 5-LOX cascade are LT₄ and the cysteinyl LTs (LT₄, LTD₄, and LTE₄), which are derived from the unstable epoxide intermediate LTA₄ (12). LT₄ is a potent stimulator of leukocyte activation and promotes adhesion of these cells to vascular endothelium. It elicits chemokinetic and chemotactic responses and also stimulates the production and release of proinflammatory cytokines from macrophages and lymphocytes. Unlike prostaglandins, which are short-lived and synthesized only according to immediate need and then rapidly degraded, LTs are quite stable, with a half-life approaching 4 hours (9). LTs have been reported to play a prominent inductive role in the progression of a variety of cancers, with 5-LOX found to be expressed in lung, colon, breast, prostate, pancreatic, and other cancers (13–19). Recent studies have also demonstrated expression of the 5-LOX pathway enzyme LTA₄ hydrolase in esophageal cancers (20). In murine models, the efficacy of 5-LOX inhibitors in preventing lung tumorigenesis was examined in A/J mice that were given the tobacco-specific carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone. The 5-LOX inhibitor A-79175 reduced lung tumor multiplicity and tumor incidence by 75% and 20% (1), respectively. 5-LOX pathway metabolites have also been implicated in promoting tumor neoangiogenesis (10).
It has long been speculated that inhibition of COX may lead to a shunting of the AA metabolism toward the production of 5-LOX pathway-derived LTs. Most of the studies to date, however, have not addressed the impact of COX-2 inhibition on the in vivo synthesis of 5-LOX-derived metabolites. We therefore investigated the effect of oral celecoxib (Celebrex) treatment on LTB4 production in the lungs of active smokers, as part of a pilot study to evaluate the feasibility of celecoxib as a chemopreventive agent for lung cancer. Furthermore, because ongoing inflammation may contribute to the process of carcinogenesis (21) and lipopolysaccharide (LPS) is a proinflammatory stimulus known to modulate the production of AA metabolites by mononuclear cells (22, 23), we also evaluated the effect of LPS on LTB4 production by human AMs with or without COX-2 inhibition.

MATERIALS AND METHODS

Celecoxib Clinical Study Design. Active heavy smokers ≥45 years of age, with a smoking history of at least 20 pack-years, were recruited and treated with oral celecoxib (Celebrex; Pfizer, New York, NY) at a dose of 400 mg twice daily for 6 months. Written informed consent was obtained in accordance with the University of California Los Angeles Institutional Review Board. Patients were screened with chest X-ray and fluorescence (LIFE) bronchoscopy (Xillix, Vancouver, Canada) to rule out the presence of lung cancer. Only subjects without evidence of lung cancer at baseline who met all entry criteria (22) were enrolled. All patients underwent repeat white light bronchoscopy with bronchoalveolar lavage (BAL) at 4 weeks after starting treatment. Bronchoscopy with BAL was also performed in a group of heavy ex-smokers with similar characteristics (≥45 years of age with at least a 30 pack-year history of smoking and smoking cessation of at least 1 year, with negative serum cotinine level) to provide control alveolar macrophages (AMs) for comparison studies.

Bronchoalveolar Lavage and In vitro Stimulation of Alveolar Macrophages. Subjects were prepped with a combination of topical anesthesia (20% benzocaine spray to pharynx plus 2% topical lidocaine as needed) and conscious sedation using midazolam and meperidine according to institutional guidelines. A fiberoptic videobronchoscope (Pentax, Inglewood, CO) was advanced into the airway and wedged into a subsegment of the right middle lobe. Four 60-mL aliquots of room temperature saline were serially lavaged and recovered by manual syringe suction. Recovered fluid was passed through a 100-μm sterile nylon filter (Becton Dickinson, San Jose, CA) to remove mucus and particulates, pooled, and centrifuged at 300 × g for 8 minutes at 4°C. The BAL fluid was then harvested, aliquoted, and stored at −80°C until analysis.

LTB4 production was stimulated by two methods. For calcium ionophore stimulation, BAL cell pellets were first washed in Hank’s balanced salt solution (Sigma-Aldrich, St. Louis, MO) and then resuspended in X-Vivo 15 serum-free medium (BioWhittaker, Walkersville, MD) to a concentration of 5 × 106 cells/mL. Cells were incubated with 2 μmol/L A23187 (Calbiochem, San Diego, CA) for 30 minutes, and the culture supernatants were harvested and stored at −80°C until analysis. BAL cells were also washed once in PBS (Irvine Scientific, Santa Ana, CA) and resuspended in X-Vivo 15 serum-free medium (BioWhittaker) to a concentration of 0.5 × 106 cells/mL. Control cells and cells stimulated with 5 μg/mL LPS (Escherichia coli 026:B6; Sigma-Aldrich) in the presence or absence of SC58236, a selective COX-2 inhibitor belonging to the same 1,5-diarylpyrazole class of COX-2 inhibitors as celecoxib (ref. 24; 800 ng/mL; Pfizer) at 37°C. In a similar set of experiments, in addition to the above culture conditions, BAL cells obtained from ex-smokers were treated with varying concentrations of the 5-LOX inhibitor Zileuton (1 ng/mL to 5 μg/mL; Abbott Laboratory, North Chicago, IL) and cyclooxygenase (10 μg/mL; Sigma-Aldrich), with or without 5 μg/mL LPS and 1.5 μg/mL SC58236. BAL cells obtained from ex-smokers were also exposed to smoke from 1 cigarette (regular Marlboro; Philip Morris, Richmond, VA) for 5 minutes using the smoking chamber (Billups-Rothenberg, Del Mar, CA) as described previously (25), followed by 24-hour stimulation with LPS in the presence or absence of SC58236. Duplicate sets of BAL cell cultures were set up simultaneously in a separate chamber containing room air as a control. The conditioned supernatants and total RNA were harvested after 24 hours of incubation and stored at −80°C until analysis. Stock solutions of SC58236 and Zileuton were prepared at a concentration of 1 mg/mL and 0.3 mg/mL in dimethyl sulfoxide, respectively, and stored at −80°C.

Isolation of Messenger RNA from Bronchoalveolar Lavage Cells and Real-Time Polymerase Chain Reaction. Total RNA from freshly harvested BAL cells and conditioned AMs was isolated using RNAsesy miniprep kits according to the manufacturer’s instructions (Qiagen, Valencia, CA.). First-strand cDNA was synthesized using 2.5 μg of total RNA (DNase-treated) in a 50-μL reverse transcriptase reaction mixture from the iScript cDNA Synthesis Kit (Bio-Rad, Hercules, CA). A region of β-actin, 5-LOX, and FLAP mRNA was amplified using specific primer pairs: (a) β-actin, 5′-GTACACTG-GCATCCTGTTAGT-3′ (sense) and 5′-ATCTTCTCAGGAGGATCTCA-3′ (antisense); (b) 5-LOX, 5′-AAGTGCCCAGA-GATGAC-3′ (sense) and 5′-GTCTCACCCTCATCCTACG-3′ (antisense); and (c) FLAP, 5′-GACACCTGTGCATCATATT-3′ (sense) and 5′-TATGTAGTTTCAAGTGACTTCC-3′ [antisense (IDT, Coralville, IA)]. The length of the cDNA amplified fragments are as follows: β-actin, 136 bp; 5-LOX, 96 bp; and FLAP, 119 bp. All real-time polymerase chain reactions were performed in a 25-μL mixture containing 1:25 volume of cDNA preparation (2 μL) and 1× iQ SYBR Green Supermix (Bio-Rad). The polymerase chain reaction amplification protocol consists of 1-minute denaturing (95°C), 1-minute annealing (60°C), and 1-minute extension (72°C) for a total of 35 cycles. Real-time quantifications were performed using the Bio-Rad iCycler iQ system (Bio-Rad). The fluorescence threshold value was calculated using the iCycler iQ system software (polymerase chain reaction efficiency ranged from 90% to 97%).

Measurement of Leukotriene B4, Prostaglandin E2, and Albumin. LTB4 concentrations in BAL and from conditioned AMs were measured by enzyme immunoassay using a LTB4 enzyme immunoassay kit (Cayman Chemical, Ann Arbor, MI). Absorbance was determined at 405 nm by Molecular Devices Microplate Reader (Sunnyvale, CA). To control for interprocedure variability associated with BAL, levels of albumin were
also measured in BAL fluid using an albumin enzyme-linked immunosorbent assay kit (Bethyl Laboratory, Montgomery, TX). The final LTB4 concentrations in BAL were then corrected using the LTB4/albumin ratio.

Statistical Analysis. The effects of oral celecoxib on intrapulmonary LTB4 production were determined by comparing baseline BAL levels with those obtained at 1 month after treatment using paired t tests. Similarly, the effects of celecoxib, Zileuton, and LPS on the production of LTB4 by cultured AM cells were determined by paired t tests. Batch analyses were performed for each subject/comparison group to eliminate interassay variability.

RESULTS

Oral Celecoxib Treatment Increases LTB4 Levels in BAL Fluid in Active Smokers, without Changing the Expression of 5-LOX or FLAP mRNA in BAL Cells. To determine the effect of oral celecoxib therapy on the release of LTB4 into the lung microenvironment, BAL fluid was obtained from active smokers before celecoxib treatment and after 1 month of treatment with oral celecoxib. Celecoxib increased LTB4 levels in BAL by an average of 5 pg/mL or 36%, or by 61% when normalized to albumin concentration (Fig. 1A and B; mean ± SEM; P < 0.03; n = 9, respectively). However, oral celecoxib treatment did not induce the expression of 5-LOX and FLAP mRNA in BAL cells (data not shown).

![Graph A](image1.png)  
**Fig. 1**  Oral celecoxib treatment increases LTB4 levels in BAL fluid in active smokers, without changing the expression of 5-LOX or FLAP mRNA in BAL cells. BAL fluid was obtained from active smokers before celecoxib treatment and after 1 month of treatment with oral celecoxib. Celecoxib increased LTB4 levels in BAL by 36% (expressed as pg/mL, A) or 61% normalized to albumin concentration (B; mean ± SEM; *, P < 0.03; n = 9, respectively).

Oral Celecoxib Treatment Increases the Capacity for AMs to Produce Leukotriene B4 in Active Smokers. We then evaluated the effect of celecoxib therapy on the calcium ionophore-stimulated production of LTB4 by ex vivo BAL cells. As reported previously, BAL cells collected from active smokers consisted of >90% AMs (26). Freshly isolated AM cells were collected from three subjects before celecoxib treatment and after 1 month of oral celecoxib treatment. Stimulation of AMs obtained at baseline (before celecoxib treatment) with A23187 (2 μmol/L) for 30 minutes resulted in a 1.75-fold increase in LTB4 production. In contrast, stimulation of AMs obtained from subjects after 1 month of celecoxib treatment resulted in a 10.6-fold increase in LTB4 production, as compared with baseline (mean ± SEM; *, P < 0.01; n = 3).

![Graph B](image2.png)  
**Fig. 2**  Oral celecoxib treatment increases the capacity for AMs to produce LTB4 in active smokers. Freshly isolated AM cells were collected from three subjects before celecoxib treatment and after 1 month of oral celecoxib treatment. Stimulation of AMs obtained at baseline (before celecoxib treatment) with A23187 (2 μmol/L) for 30 minutes resulted in a 1.75-fold increase in LTB4 production. In contrast, stimulation of AMs obtained from subjects after 1 month of celecoxib treatment resulted in a 10.6-fold increase in LTB4 production, as compared with baseline (mean ± SEM; *, P < 0.01; n = 3).

In vitro Treatment with LPS Significantly Induced the Production of LTB4 by AMs Recovered from Untreated Active Smokers and Ex-Smokers. To further evaluate the responsiveness of AMs, BAL cells obtained from untreated active and ex-smokers were conditioned in vitro for 24 hours with LPS (5 μg/mL), in the presence or absence of the COX-2 inhibitor SC58236 (800 ng/mL). Stimulation with LPS induced a 9.6-fold increase in LTB4 production by smokers’ AM (Fig. 3; mean ± SEM; P < 0.05; n = 8). SC58236 treatment, however, did not significantly alter the constitutive or LPS-induced LTB4 production. Interestingly, AMs obtained from ex-smokers were less responsive to LPS stimulation, with an observed 4-fold increase in response to LPS conditioning (Fig. 4A; mean ± SEM; P < 0.05; n = 8) versus the 9.6-fold increase in smokers’ AMs. These data suggest that AMs from active smokers are primed to produce higher levels of LTB4 when exposed to inflammatory stimuli, such as LPS. To further explore whether cigarette smoke induces LTB4 production, BAL cells from ex-smokers were first exposed to cigarette smoke for 5 minutes, followed by 24 hours of LPS (5 μg/mL) stimulation, with or without SC58236 (800 ng/mL). Smoke exposure increased LTB4 pro-
Inhibition of 5-LOX Dose-Dependently Blocks LPS-Induced LTB₄ Production by AMs from Ex-Smokers. We then evaluated the effect of 5-LOX inhibition with Zileuton, a 5-LOX–specific inhibitor. At 5 µg/mL, Zileuton completely abolished the LPS-induced production of LTB₄ by AMs obtained from untreated ex-smokers, indicating that the LPS-induced increase in LTB₄ production is mediated via the 5-LOX pathway (Fig. 5A; mean ± SEM; *, P < 0.05; n = 5). At doses ranging from 1 to 500 ng/mL, Zileuton abrogated the LPS-induced production of LTB₄ in a dose-responsive manner, suggesting that the LPS effect is due to an increase in 5-LOX enzymatic activity (Fig. 5B; mean ± SD; P < 0.05; n = 2).

Cycloheximide Increases LTB₄ Production and Simultaneously Decreases LPS-Induced PGE₂ Synthesis in AMs from Ex-Smokers. To determine whether LPS-induced LTB₄ is mediated through the action of protein intermediates, cycloheximide (10 µg/mL) was added to similarly conditioned AM cultures to inhibit protein synthesis. Interestingly, inhibition of protein synthesis markedly increased LTB₄ in all conditions (Fig. 6A) but abrogated the LPS-induced increase in PGE₂ synthesis (Fig. 6B), suggesting a shunting phenomenon and/or LPS-associated induction of newly synthesized protein inhibitors of the 5-LOX metabolic pathway. These data also confirmed that the LPS-induced LTB₄ production by AMs did not occur as a result of increased 5-LOX or FLAP protein synthesis (Fig. 6; mean ± SD; P < 0.05; n = 2).

DISCUSSION
Metabolites derived from the 5-LOX pathway have been extensively implicated in the pathogenesis of a variety of diseases, such as allergic rhinitis, asthma, chronic obstructive pulmon...
COX-2 Inhibitors Modulate Pulmonary LTB₄ Production

óngmonary disease, scleroderma lung disease, and malignancies, including that of lung cancer (1, 14, 27–30). To determine the effect(s) of COX-2 inhibitors on the 5-LOX signaling pathway in the lung microenvironment of active and former smokers, we evaluated the modulated release of LTB₄ under in vivo, ex vivo, and in vitro conditions. One month of oral celecoxib treatment resulted in a modest increase in LTB₄ levels in BAL fluid obtained from active smokers. Because AMs are the predominant effector cells in the lung microenvironment and are the major source of LTB₄ production in lung lining fluid, we also examined the specific generation of LTB₄ by this cell population. We found that 1 month of oral celecoxib treatment markedly increased the capacity for LTBA release from ex vivo AMs in active smokers in response to calcium ionophore stimulation. To further characterize the effect in vitro, AMs obtained from smokers and ex-smokers were treated overnight with the COX-2 inhibitor SC58236, with or without LPS stimulation. Prolonged high-dose LPS exposure (at 5 μg/mL over 24 hours) significantly increased LTB₄ generation by AMs obtained from both active and ex-smokers; however, the degree of LPS induction was notably attenuated in ex-smokers. In addition, concomitant COX-2 inhibition with SC58236 did not significantly impact these changes. Inhibition of the 5-LOX enzyme resulted in a dose-dependent decrease in the induction of LTB₄ synthesis by LPS. Cycloheximide studies indicate that new protein synthesis is not required for the observed LPS induction of LTB₄ generation. However, it is required for the LPS induction of PGE₂ synthesis.

Our findings indicate that oral celecoxib therapy results in alterations of LTB₄ release in the lung microenvironment of active smokers. Although our studies noted relatively low basal LTB₄ levels in BAL fluid, as such, whereas the observed average increase of 5 pg/mL (15 pmol/L) in the BAL fluid is statistically significant, the actual functional significance of such a relatively small increase is unclear. Indeed, previous studies indicate that a substantially higher concentration of LTB₄ (390 pmol/L) is required to induce chemotaxis and chemokinesis in human polymorphonuclear cells (31). Studies of 5-LOX and FLAP mRNA expression in BAL cells indicate that the mechanism of this induction involves posttranscriptional events because mRNA levels were not increased by oral celecoxib therapy. Furthermore, we demonstrate that oral celecoxib treatment markedly increased the responsiveness of ex vivo smokers’ AMs to calcium ionophore stimulation. In previous reports, we showed that oral administration of celecoxib significantly abrogated the capacity for calcium ionophore to stimulate PGE₂ synthesis in AMs obtained from active smoker (22). Collectively, these data suggest that COX-2 inhibition functions to phenotypically favor the synthesis of 5-LOX pathway LTs over that of COX-2–derived prostaglandins in AMs, perhaps via a shunting mechanism. However, the possibility that COX-2 inhibitors may alter other posttranslational events that may, in turn, affect the turnover/synthesis rate of 5-LOX or FLAP cannot be fully excluded.

To determine the effect of inflammatory stimuli that are potentially tumorigenic and known to modulate activity of the 5-LOX pathway in monocytes/macrophages (22, 23), we further examined the responsiveness of AMs obtained from active and ex-smokers to LPS. Because mechanisms of inflammation associated with recurring infections may facilitate tumorigenesis (32), it is of great interest to determine whether or not COX-2 inhibition alters AM LTB₄ production when the cells are exposed to LPS. We demonstrate that prolonged high-dose LPS exposure (at 5 μg/mL) increases acute LTB₄ generation by AMs obtained from both active and ex-smokers. Interestingly, this LPS induction was comparatively attenuated in AMs obtained from ex-smokers, suggesting that ongoing in vivo tobacco smoke exposure may prime the 5-LOX pathway for responsiveness to inflammatory stimuli. This notion is further supported by the fact that in vitro smoke exposure also significantly increased LTB₄ production by ex-smokers’ AMs. Such observations suggest that the LT-related responsiveness of AMs to bacterial respiratory infection is modulated by ongoing smoke exposure. However, this hypothesis requires further investigation.

Overnight COX-2 inhibition in vitro, however, did not significantly increase LTB₄ levels or LPS responsiveness in both smokers’ and ex-smokers’ AMs. These findings seem inconsistent with the in vivo data, suggesting that mechanisms other than shunting account for the increase of LTB₄ in response to LPS. It is conceivable that shunting is the predominant mechanism involved in the setting of acute calcium ionophore stimulation, when 5-LOX enzymatic activity is suddenly and highly up-regulated, whereas under normal physiologic conditions of calcium homeostasis or in the presence of LPS, shunting may play a minor role in increasing LTB₄ production in the lung microenvironment in the presence of a COX-2 inhibitor. This could explain the modest increase of LTB₄ in the posttreatment
BAL, in contrast to the many fold increase in AM LTB₄ production with calcium ionophore stimulation. This notion is further supported by the fact that in vivo celecoxib treatment did not alter 5-LOX or FLAP mRNA expression in smokers’ AMs (data not shown). To our knowledge, this is the first report demonstrating that oral celecoxib, at a dose of 400 mg twice daily, is capable of modulating LTB₄ synthesis within the lung microenvironment of active smokers in vivo.

LTB₄, or endotoxin, is a component of Gram-negative bacteria cell wall known to induce inflammation and potent immune responses that can contribute to host cell damage and lead to sepsis syndrome. Studies suggest that acute LPS exposure induces the synthesis of LTB₄ by lung tissue (33, 34) and the development of pulmonary neutrophilia (35). Previous studies have shown that LPS modulates the release of AA metabolites and other inflammatory mediators from monocytes/macrophages (36, 37). LPS is known to increase AA release from membranes (38–40). Acute LPS exposure has also been shown to prime alveolar macrophages for LTB₄ release (36, 37). However, the LPS-induced modulation of human AM LTB₄ production is currently incompletely understood. In this study, we demonstrate that LPS induced the production of LTB₄ by human AMs and that such an effect is sustained at 24 hours. To further delineate the mechanisms responsible for LPS-induced LTB₄ production in AMs, we co-conditioned AMs with the 5-LOX inhibitor Zileuton. At 5 μg/mL, a concentration typically achieved by oral administration, Zileuton completely abrogated the LPS-induced LTB₄ production, indicating that the induction is mediated via the 5-LOX pathway.

There are several possibilities whereby LPS may induce LTB₄ production in human AMs. The sustained increase in LTB₄ at 24 hours may be due to an increased production of 5-LOX, FLAP, or both or through an increased synthesis of other protein intermediates. Alternatively, it may be mediated through augmentation of 5-LOX enzymatic activity. Intriguingly, rather than abrogating the LPS-induced LTB₄ production, inhibition of protein synthesis with cycloheximide markedly increases LTB₄ in all conditions and simultaneously abrogates LPS-induced PGE₂ production. Because LPS-induced PGE₂ synthesis by human AMs is primarily mediated through up-regulation of COX-2 (22), a plausible explanation is that cycloheximide inhibits COX-2 production and therefore allows most of the AA precursors to be shunted toward the LT pathway. Furthermore, additional shunting of AA may come from metabolic pathways other than COX-2 that are also induced by LPS. The fact that COX-2 inhibitors failed to induce LTB₄ as did cycloheximide also supports our aforementioned “threshold” concept because pharmacological COX-2 inhibitors usually do not completely inhibit COX-2 activity. Thus, in order for shunting to become significant in the induction of LTB₄ synthesis, the amount of AA precursors may need to exceed a certain threshold, within a specific time frame. Alternatively, LPS could induce the synthesis of inhibitory intermediary proteins that suppress 5-LOX and FLAP function, such as nitric oxide synthase, which has been described previously to inhibit 5-LOX metabolism in rat AMs (23). In any event, the cycloheximide experiments confirm the finding that LPS-induced LTB₄ synthesis is not mediated through up-regulation of 5-LOX or FLAP protein production.

We also demonstrate that Zileuton abrogates LPS-induced LTB₄ in a dose-responsive manner, suggesting that the increase in LTB₄ synthesis is due to an increase in 5-LOX enzymatic activity. Exactly how LPS increases 5-LOX enzymatic activity in AMs is unclear. Possible mechanisms for LPS induction of 5-LOX activity may involve increased availability of AA substrate or increased intracellular calcium mobilization. Because we did not measure the total release of AA, the possibility that LPS may increase substrate release at the level of phospholipase cannot be excluded.

The association between mechanisms of inflammation and risk for cancer is becoming increasingly recognized. Inflammatory-mediated events, such as the production of reactive oxygen species, the activation of growth factors in wound repair, and the alteration of signal transduction to activate cell proliferation for replacement of necrotic tissue and cells, are all mechanisms known to occur during multistep carcinogenesis (32). Central to this concept is the involvement of AA metabolites. Indeed, the aberrant production of these biological response modifiers appears to play a major role in conferring the malignant phenotype. With the growing use of COX-2 inhibitors both as anti-inflammatory agents for treatment of arthritis or pain and as potential adjuvant antineoplastic or chemopreventive agents, the consequence of COX-2 inhibition on the formation of other AA metabolites such as LTB₄ is an obvious concern. In this report, we demonstrate that whereas COX-2 inhibition may lead to shunting of AA toward the LT pathway, the contribution from shunting is comparatively small under normal physiologic conditions. In addition, even in the setting of proinflammatory stimulation with LPS, the presence of a COX-2 inhibitor did not significantly alter the induction of LTB₄, although it should be noted that AMs from three subjects did show a modest increase. The exact mechanisms responsible for this heterogeneous responsiveness are unclear and could potentially contribute to COX-2 resistance with prolonged use. Nevertheless, our findings suggest that in most cases, COX-2 inhibition does not significantly perturb the 5-LOX/LTB₄ signaling pathways in the lungs and support the continued investigation of celecoxib as an antineoplastic, chemopreventive agent.

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