Effects of selective COX-2 inhibition on allergen-induced bronchoconstriction and airway inflammation in asthma

Kameran Daham, MD, PhD,[a,b] Anna James, PhD,[b,c] David Balgoma, PhD,[b,c] Maciej Kupczyk, MD, PhD,[b,c] Bo Billing, MD, PhD,[a,b] Agneta Lindeberg, RN,[a,b] Elisabeth Henriksson, BMA,[a,b] Garret A. FitzGerald, MD, PhD,[d] Craig E. Wheelock, PhD,[b,e] Sven-Erik Dahlén, MD, PhD,[b,c] and Barbro Dahlén, MD, PhD[a,b] *Stockholm, Sweden, and Philadelphia, Pa*

Background: Prostaglandins that constrict and relax airways are synthesized in reactions catalyzed by either COX-1 or COX-2. It is not known whether selective inhibition of COX-2 makes asthmatic responses better or worse.

Objective: To determine the effects of the selective COX-2 inhibitor, etoricoxib, on allergen-induced bronchoconstriction in asthmatic subjects.

Methods: Sixteen subjects with mild atopic asthma underwent rising dose inhalation challenges with allergen or methacholine to determine PD_{20} FEV_1 during a control study period or after 10 to 13 days of treatment with etoricoxib (90 mg once daily). The order of study periods was randomized with at least 2-week washout periods. Induced sputum cells and fractional exhaled nitric oxide levels were used to assess airway inflammation and blood assays for COX-1 and COX-2 activity to assess enzyme inhibition. Urinary excretion of lipids was used to assess prostaglandin biosynthesis.

Results: Etoricoxib did not change baseline lung function, nor airway responsiveness to allergen or to methacholine. Neither were the allergen-induced increase in sputum eosinophils and fractional exhaled nitric oxide levels affected by treatment. The biochemical effectiveness of the treatment was established both in the blood assays and by the distinct inhibitory effect of etoricoxib on urinary excretion of tetranor-prostaglandin E_2 ($P < .001$).

Conclusions: This first study of COX-2 inhibition in provoked asthma found no negative effects of etoricoxib on allergen-induced airflow obstruction and sputum eosinophils, basal lung function, or methacholine responsiveness. The study suggests that short-term use of COX-2 inhibitors is safe in subjects with asthma. (J Allergy Clin Immunol 2014;134:306-13.)

Key words: *Asthma, COX, NSAIDs, eicosanoids, prostaglandin, thromboxane, leukotrienes, airway hyperresponsiveness, mass spectrometry, urinary metabolites*

Prostaglandins are lipid mediators of inflammation and capable of both constricting and relaxing the airways.[1] Prostaglandins are synthesized by either of 2 COX isoenzymes, the constitutively expressed COX-1, active under basal conditions, or COX-2, which is often induced during inflammation.[1] Although traditional nonsteroidal anti-inflammatory drugs (NSAIDs) block both COX isoforms, the coxibs preferentially inhibit COX-2.

In the current study, we hypothesized that the inhibition of COX-2 might be associated with an exaggerated airway response to allergen challenge, by decreasing formation of the bronchodilator prostaglandin E_2 (PGE_2)[2] while maintaining production of the bronchoconstrictor prostaglandin D_2 (PGD_2).[3] This hypothesis was based on the results of a previous study in which we found that basal biosynthesis of PGD_2 was increased in asthmatic subjects and its formation was catalyzed exclusively by COX-1.[4] In contrast, the same study found that COX-2 contributed substantially (>65%) to whole-body PGE_2 biosynthesis. Although PGE_2 is predominantly considered to be proinflammatory in most parts of the body, it appears to have mainly protective and anti-inflammatory effects in the airways. In mouse models of asthma, it has been suggested that the inhibition of COX-2 leads to enhanced airway hyperresponsiveness.[5,6] In subjects with asthma, inhalation of prostaglandin E (PGE) inhibits the release of cysteinyl-leukotrienes and attenuates the bronchoconstriction induced by allergen and other indirect stimuli.[2,7-9] Our primary aim was therefore to examine whether COX-2 inhibition triggers a proasthmatic imbalance in the prostaglandin system, by blocking the protective PGE_2 while maintaining the biosynthesis of disease-driving PGD_2.

The clinical relevance of this study was therefore to provide information concerning the safety of COX-2 inhibitors in patients with asthma. Although coxibs are unsuitable for chronic treatment of inflammation due to cardiovascular side effects,[1] they are helpful in the short-term alleviation of acute episodes of pain or inflammation due to minimal effects on bleeding and gastrointestinal integrity. It was therefore considered important to assess the effects of short-term use of COX-2 inhibitors in asthmatic subjects in a setting in which experimental worsening of asthma was induced, especially as asthmatic subjects in general are often advised to avoid NSAIDs because of the potentially life-threatening reactions that may occur in patients with a well-defined subphenotype of asthma, aspirin-exacerbated

From [a]the Department of Medicine Huddinge, [b]the Centre for Allergy Research, [c]the Institute of Environmental Medicine, and [e]the Department of Medical Biochemistry and Biophysics, Karolinska Institutet, Stockholm; and [d]the University of Pennsylvania, Philadelphia.

This study was supported by the Swedish Heart-Lung Foundation, the Swedish Medical Research Council, Vinnova (CiDAT), The Stockholm County Council Research Funds (ALF), the Asthma and Allergy Research Foundation, The Centre for Allergy Research, and Karolinska Institutet. A.J. and M.K. were supported by the Bernard Osher Initiative for research on severe asthma, and D.B. was supported by the Unbiased BIOmarkers in PREDiction of respiratory disease outcomes project (Innovative Medicines Initiative for severe asthma).

Disclosure of potential conflict of interest: The authors have received research support from the Swedish Medical Research Council, the Swedish the Heart-Lung Foundation, the Stockholm County Council Research Funds, and Vinnova.

Received for publication July 10, 2013; revised November 4, 2013; accepted for publication December 3, 2013.

Available online January 22, 2014.

Corresponding author: Kameran Daham, MD, PhD, Department of Respiratory Medicine and Allergy, M53, Karolinska University Hospital, Huddinge, SE-141 85 Stockholm, Sweden. E-mail: kameran.daham@ki.se.

0091-6749/$36.00

http://dx.doi.org/10.1016/j.jaci.2013.12.002

Abbreviations used
FENO: Fractional exhaled nitric oxide
NSAIDs: Nonsteroidal anti-inflammatory drugs
PGD_2: Prostaglandin D_2
PGE: Prostaglandin E
PGE_2: Prostaglandin E_2
$PGF_{1\alpha}$: Prostaglandin $F_{1\alpha}$
PGI_2: Prostaglandin I_2
TXA_2: Thromboxane A_2
TXB_2: Thromboxane B_2

respiratory disease. In these patients, exacerbations are precipitated by the intake of aspirin and other NSAIDs that inhibit COX enzymes.[10] In view of the many indications for NSAIDs in the treatment of inflammation, fever, and pain, this recommendation is both unfortunate and probably often unnecessary. While there is good evidence that coxibs are tolerated by patients with aspirin-exacerbated respiratory disease, at least when they are clinically stable,[11-13] the safety of COX-2 inhibitors in subjects with asthma in general has not been established.

In this study, the effect of COX-2 inhibition on induced asthmatic airway obstruction and inflammation was therefore investigated by intervention with one of the most specific COX-2 inhibitors, etoricoxib.[14] Bronchial allergen challenges were performed to produce a graded and standardized airway obstruction. This is a safe way to mimic a naturally occurring exacerbation with a well-established clinical relevance.[15] Fractional exhaled nitric oxide (FENO) and eosinophils in induced sputum were analyzed as biomarkers of altered asthmatic airway inflammation. To confirm the effectiveness and selectivity of COX inhibition, standard functional blood assays were used.[16] Finally, the effect of the allergen challenge and COX-2 inhibition on the biosynthesis of prostanoids was determined by using mass spectrometric analysis of urinary prostaglandin metabolites.[17]

METHODS

Subjects

Sixteen nonsmoking subjects, aged 18 to 55 years, with mild atopic asthma, an FEV_1 of 75% or more of predicted normal, and a positive methacholine bronchoprovocation were recruited from our clinic and via advertisement. The subjects were treated only with short-acting bronchodilators and had no respiratory disease other than asthma, and no respiratory infection in the 4 weeks before inclusion. Exclusion criteria included hypersensitivity to coxibs, and a history of cardiovascular disease, including hypertension. The use of paracetamol, NSAIDs, and other anti-inflammatory drugs was not allowed during the study.

The Ethical Review Board in Stockholm approved the study (Dnr 2009/959-31-4, 2009/1422-32), and the subjects gave written informed consent.

Study design

The study comprised a screening phase followed by a randomized 2-period, cross-over comparison between active treatment with the selective COX-2 inhibitor, etoricoxib, and an untreated control period with identical design (Fig 1). Those carrying out analysis of sputum and biochemical end points were unaware of which period the active treatment was given.

At screening, data on baseline characteristics including FENO, FEV_1, skin prick testing, specific IgE for the allergen selected to be used in the challenges, and current airway sensitivity to methacholine and allergen were obtained (Table I). A washout of at least 14 days preceded the start of the cross-over phase, which comprised 3 clinic visits during each period (Fig 1). Etoricoxib (Arcoxia; Merck Sharp & Dohme, South Granville, Australia) tablets 90 mg were purchased from the hospital pharmacy and administered once daily for 10 to 13 days, with the first dose taken in the clinic after baseline assessments on study day 1 of the treatment period. A methacholine challenge was performed on the first and the penultimate day of each period, and an allergen inhalation challenge was then performed on the last day of each period. Sampling of blood was performed at baseline (study day 1), and on the last 2 days of each period (study days 2 and 3, 1 hour before methacholine and allergen challenges, respectively). Sputum induction was performed 1 hour after methacholine challenge on study days 1 and 2 and at 6 hours after the maximum fall in FEV_1 following allergen provocation on study day 3 (Fig 1). Urine was collected before the start of allergen broncho-provocation, and at 1 and 2 hours after the maximum fall in FEV_1.

Bronchoprovocations

Inhalation challenges were performed as previously described[18] by using a dosimeter-controlled jet nebulizer (Spira Electro 2; Respiratory Care Center, Hameenlinna, Finland) and with pulmonary function measured as FEV_1. Allergen and its diluent (Aquagen) were purchased from ALK Laboratories (Copenhagen, Denmark) and methacholine from Norrland's University Hospital Pharmacy (Umea, Sweden). Challenges were always performed in the morning and started by inhalation of the diluent. Provided the FEV_1 did not change by more than 10%, inhalation of methacholine or the allergen to which the subjects were sensitized was commenced with the postdiluent FEV_1 value used as baseline. Half-log increments in the cumulated dose of allergen were inhaled every 15 minutes (7-7100 SQ units), whereas methacholine was administered every third minute in doubling doses (14.2-7256 μg). The challenge was stopped when the FEV_1 had fallen by at least 20%. The PD_{20} value was derived by linear interpolation from the log cumulated dose-response curve.

Measurement of FENO

FENO (NIOX analyzer; Aerocrine AB, Solna, Sweden) was measured at a flow rate of 50 mL/s according to American Thoracic Society guidelines.[19]

Sputum induction

Briefly, subjects inhaled 0.2 mg albuterol and provided the FEV_1 was 70% or more of predicted inhaled an aerosol (DeVilBiss Ultraneb 3000; Dolema AB, Täby, Sweden) containing increasing concentrations of saline (3%, 4%, and 5%) for 7 minutes each.[20] Spirometry was obtained after each concentration, and the induction was stopped only if the FEV_1 declined by 20%. Sputum plugs were extracted from the sample and processed within 2 hours as described.[20] Cell viability was assessed by using trypan blue solution (0.4%), and cells were classified as viable, nonviable, and squamous; the accepted proportion for the latter was less than 20%. Cytospins were stained with May-Grünwald-Giemsa solution, and total and differential nonsquamous cell counts were performed.

COX-1 and COX-2 assays

COX-1 activity was assessed by thromboxane B_2 (TXB_2) generation in clotted blood (1 hour, 37°C), and COX-2 activity was assessed by the formation of PGE_2 in heparinized blood stimulated with LPS (100 μg/mL; 24 hours, 37°C), as previously described,[16] using enzyme-immunoassays (Cayman Chemical, Ann Arbor, Mich). The biological activity of etoricoxib was validated by the addition of the drug *ex vivo* (0.1-10 μM) to blood drawn from the subjects during the control arm of the study. Results confirmed a dose-dependent inhibition of LPS-induced PGE_2 formation with an IC_{50} of 0.78 μM (Table II). Conversely, it was documented that the addition of etoricoxib *ex vivo* had no effect on TXB_2 levels (Table II).

Collection of urine and measurement of urinary metabolites

Urine was stored at −70°C until assayed. Metabolites of prostaglandins, thromboxane, and leukotriene E_4 were measured by the use of ultra