

COX-2 Inhibition Attenuates Cough Reflex Sensitivity to Inhaled Capsaicin in Patients With Asthma

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■ Abstract

Background and objective: Cyclooxygenase (COX) is an enzyme that converts arachidonic acid to prostanoids. There are two isoforms of COX, namely COX-1 and COX-2. COX-2 is highly inducible by several stimuli and is associated with inflammation. Recent studies have shown that COX-2 is upregulated in the airway epithelium of patients with asthma but little is known about the role it plays in cough, a common symptom of bronchial asthma. This study was designed to investigate the role of COX-2 in cough reflex sensitivity in patients with asthma.

Patients and methods: The effect of etodolac, a potent COX-2 inhibitor, on cough response to inhaled capsaicin was examined in 17 patients with stable asthma in a randomized, placebo-controlled crossover study. Capsaicin cough threshold, defined as the lowest concentration of capsaicin eliciting 5 or more coughs, was measured as an index of airway cough reflex sensitivity.

Results: The geometric mean (geometric SEM) cough threshold was significantly increased after a 2-week treatment program with oral etodolac (200 mg twice a day) compared with placebo (36.7 [1.2] vs 21.6 [1.2] μ M, $P < .02$).

Conclusions: These findings indicate that COX-2 may be a possible modulator augmenting airway cough reflex sensitivity in asthmatic airways.

Key words: Airway cough reflex sensitivity. Capsaicin. Etodolac. COX-2. Bronchial asthma.

■ Resumen

Antecedentes y objetivo: La ciclooxigenasa (COX) es un enzima que convierte el ácido araquidónico en prostanoideos. Hay dos isoformas de COX, COX-1 y COX-2. COX-2 es altamente inducible por diversos estímulos y está asociado a la inflamación. Estudios recientes han demostrado que la COX-2 se sobreexpresa en el epitelio respiratorio de los pacientes con asma pero poco se conoce acerca del papel que juega en la tos, un síntoma común del asma bronquial. Este estudio se diseñó para investigar el papel de la COX-2 en la sensibilidad del reflejo de la tos en pacientes con asma.

Pacientes y métodos: En este estudio se analiza el efecto del etodolaco, un potente inhibidor de la COX-2, sobre la tos producida en respuesta a la inhalación de capsaicina, en 17 pacientes con asma estable en un estudio cruzado aleatorizado controlado con placebo.

El umbral de tos provocada por capsaicina, definido como la concentración más baja de capsaicina que produce cinco o más toses, se mide como un índice de la sensibilidad del reflejo de la tos de las vías aéreas.

Resultados: Se observa un incremento significativo de la media geométrica del programa de dos semanas de tratamiento con etodolaco oral (200 mg, dos veces al día) en comparación con el placebo (36.7 [1.2] vs 21.6 [1.2] μ M, $P < .02$).

Conclusiones: Estos hallazgos indican que la COX-2 puede actuar como un posible modulador capaz de aumentar la sensibilidad del reflejo de la tos en las vías aéreas de los asmáticos.

Palabras clave: Sensibilidad al reflejo de la tos. Capsaicina. Etodolaco. COX-2. Asma bronquial.

Introduction

Chronic airway inflammation with eosinophils and lymphocytes is a fundamental feature of bronchial asthma [1]. There is strong evidence that arachidonic acid metabolites may play an important role in the pathogenesis of asthma [2-4]. These metabolites include 5-lipoxygenase and cyclooxygenase (COX) products. Earlier studies have revealed the existence of 2 isoforms of COX, namely COX-1 and COX-2, each with a similar molecular weight [5-6]. COX-1 is constitutively expressed and is responsible for the basal production of prostanoids, whereas COX-2 is highly inducible by a number of stimuli including cytokines and associated with inflammation. It is becoming increasingly likely that the induction and regulation of COX-2 may play a key role in the pathophysiology of several inflammatory disorders and the pathogenesis of asthma [7-9].

The above findings imply that COX-2 may be involved in controlling cough reflex sensitivity in asthma, because cough is a major symptom of asthma. Although we have shown in a previous study that the nonspecific COX inhibitor, indomethacin, can modulate airway cough reflex sensitivity to inhaled capsaicin [10], the beneficial effect of COX-2 inhibition has not been proven in a clinical study [11]. Additionally, a recent study reported the effect of COX-2 inhibition in cough reflex sensitivity in guinea pigs [12]. The aim of the present study was to analyze the effect of etodolac, a potent COX-2 inhibitor [13,14], in patients with asthma.

Patients and Methods

Patients

Seventeen patients (11 men and 6 women) with stable asthma and a mean (SEM) age of 73.6 (1.3) years (range, 39-83 years) participated in this study. All the patients were lifetime nonsmokers or exsmokers and none had had a viral infection in the 4 weeks prior to the study. Informed consent was obtained from all the patients and the study was approved by the ethics committee at our hospital.

Each of the patients satisfied the American Thoracic Society definition of asthma [15], with symptoms of episodic wheezing, cough, and shortness of breath responding to bronchodilators, and reversible airflow obstruction documented in at least 1 previous pulmonary function study. Reversibility was defined as an increase of 12% or more in forced expiratory volume in 1 second (FEV₁) following inhalation of 200 µg of salbutamol sulfate. All the patients had bronchial hyperresponsiveness. Seven of the 17 patients were classified as having atopic asthma due to increased serum levels of specific immunoglobulin (Ig) E antibodies. The other 10 patients were classified as having nonatopic asthma because they had no family history of allergic disease and did not experience an increase in specific IgE antibodies following exposure to 10 common allergens. They were taking 40 µg of short-acting oral and/or inhaled β₂-agonists (clenbuterol and procaterol, respectively), 400 to 800 µg of inhaled steroids (beclomethasone dipropionate), and/or 1500 mg of mucolytic agents (carbocysteine). They

had not received oral theophylline or oral steroid therapy for at least 8 weeks. The study was carried out when the patients' symptoms were mild and stable.

Assessment of Cough Reflex Sensitivity to Inhaled Capsaicin

Cough reflex sensitivity was assessed by capsaicin provocation testing [16]. A total of 30.5 mg of capsaicin (Sigma, St Louis, Missouri, USA) was dissolved in polysorbate 80 (1 mL) and ethanol (1 mL) and then dissolved in a saline solution (8 mL) to make a stock solution of 1×10^{-2} M, which was stored at -20°C. This solution was further diluted with saline to produce doubling concentrations ranging from 0.49 µM to 1000 µM. Each patient inhaled a control solution of saline followed by progressively increasing concentrations of capsaicin. Solutions were inhaled by tidal breathing for 15 seconds every 60 seconds; the solution was nebulized through a Bennett Twin nebulizer (3012-60cc, Puritan-Bennett Co, Carlsbad, California, USA) with an output of 0.21 mL/min; all the patients wore a noseclip. Concentrations were doubled until 5 or more coughs were elicited. The number of capsaicin-induced coughs was counted by a medical technician blinded to the treatment at the pulmonary function laboratory at our hospital. The cough threshold was defined as the lowest concentration of capsaicin that elicited 5 or more coughs, visually monitored.

Study Protocol

All concomitant medication was withdrawn at 9.00 PM on the day prior to testing to allow a washout period of at least 12 hours before the cough threshold was measured. The challenge tests were performed at 10.00 AM on each test day to reduce diurnal variability in cough response.

Each patient was tested 4 times, with an interval of 2 weeks between tests. A control measurement of the capsaicin cough threshold was carried out before the first treatment. After a 2-week washout period, patients were treated with oral etodolac and placebo in a randomized, crossover trial, also with a washout period of 2 weeks between treatments. The active drug (200 mg) and placebo were taken twice a day for 14 days and at 8.00 AM on the test day. FEV₁ was measured using a dry wedge spirometer (Transfer Test, P.K. Morgan Ltd., Rainham, UK) before the capsaicin challenge to assess the bronchoactive effect of the treatment regimens. Serum was obtained after treatment with etodolac to measure total IgE levels using the ImmunoCAP Total IgE test (Pharmacia, Uppsala, Sweden).

Statistical Analysis

Capsaicin cough threshold values were expressed as geometric mean values with a geometric standard error of the mean (GSEM). Forced vital capacity (FVC) and FEV₁ were shown as arithmetic mean values (\pm SEM). Cough threshold, FVC, and FEV₁ values were compared between each pair of the 4 test periods (run-in, placebo treatment, washout, and etodolac treatment) using the Wilcoxon signed-rank test, for which cough threshold values were transformed to logarithmic values. A *P* value of less than .05 was taken as significant.

Results

The cough thresholds to inhaled capsaicin in the 17 patients before each treatment (run-in and washout period) and after treatment with etodolac and placebo are shown in Figure 1. The geometric mean (GSEM) values for the cough threshold were 25.4 (1.2) μM in the run-in period, 25.5 (1.2) μM in the washout period, 36.7 (1.2) μM after etodolac treatment, and 21.6 (1.2) μM after placebo treatment. The cough threshold was significantly greater after etodolac treatment than after placebo treatment ($P < .02$). No significant differences were found for FVC or FEV₁ between the 4 test periods (Table).

There were no changes in serum IgE levels (Figure 2), meaning that IgE production was not altered by treatment with etodolac. None of the patients complained of cardiovascular or gastroenterological symptoms following the administration of etodolac; such symptoms have been previously reported for other COX-2 inhibitors such as rofecoxib, celecoxib, and valdecoxib [17,18].

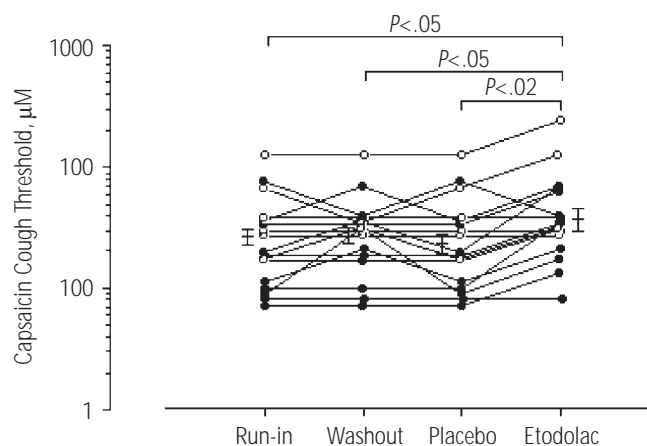


Figure 1. Individual capsaicin cough thresholds before each treatment and after placebo and etodolac treatments in patients with stable bronchial asthma (n=17). Each horizontal bar represents a geometric mean value. Closed circles and open circles represent patients with and without steroid inhalation therapy, respectively. *P* values were determined by the Wilcoxon signed-rank test using logarithmically transformed values.

Table. Pulmonary Function on Etodolac and Placebo Treatments in Patients With Bronchial Asthma

	Run-in	Washout	Placebo	Etodolac
FVC, % of predicted	105.8±4.0	107.7±4.5	107.5±3.7	104.6±4.6
FEV ₁ , % of predicted	96.7±8.0	96.7±7.4	96.0±7.1	95.2±8.0

Abbreviations: FEV₁, forced expiratory volume in 1 second; FVC, forced vital capacity.

^a Data are shown as mean ±SEM.

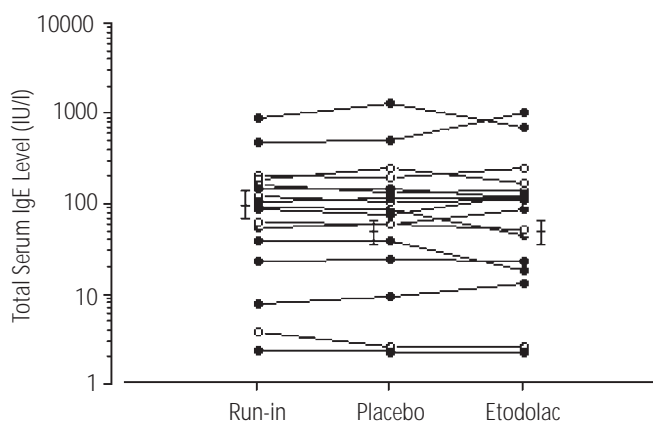


Figure 2. Individual serum immunoglobulin (Ig) E levels before each treatment and after placebo and etodolac treatments in patients with stable bronchial asthma. Each horizontal bar represents a geometric mean value. Closed circles and open circles represent patients with and without steroid inhalation respectively.

Discussion

The present study showed that treatment with a potent COX-2 inhibitor, etodolac, over 2 weeks increased the cough threshold to inhaled capsaicin in patients with stable asthma. No differences were found between patients treated with etodolac or placebo in terms of baseline pulmonary function, IgE production, or type of inflammation. Based on these findings, COX-2 may be a possible modulator augmenting airway cough reflex sensitivity in asthmatic airways, especially in the case of cough-variant asthma.

Cough is a common symptom of respiratory and nonrespiratory disorders for which patients seek medical care [19]. Although we have previously shown that inflammatory mediators play a modulatory role in airway cough reflex sensitivity to inhaled capsaicin in patients with asthma [20-22], the mechanism by which this sensitivity may be altered in humans remains unknown.

Arachidonic acid metabolites are among the mediators known to play an important role in the development of airway disease; examples of these mediators are prostaglandins, a family of metabolites from enzymes possessing COX activity [5]. Previous studies have shown that biologically active prostaglandins can be generated at

least by 2 different enzyme isoforms: COX-1 and COX-2 [5,6]. COX-1, which is constitutively expressed in most tissues and also found in blood platelets, is responsible for the production of prostaglandins. COX-1-derived metabolites are involved in cellular housekeeping functions. The inducible isoform COX-2, in contrast, is the major isoenzyme associated with inflammation; it can be induced by various stimuli, including inflammatory cytokines, leading to the further production of inflammatory substances such as prostanoids [5,6]. Elevated levels of COX-2 have been found in the epithelium and submucosa of airways of patients with asthma compared to controls [7-9], implying that COX-2 may be involved in controlling cough reflex sensitivity in asthmatic airways with chronic eosinophilic bronchial inflammation, because cough is one of the major symptoms in this disorder. Nonetheless, the role of COX-2 in the pathophysiology of cough as a major symptom of asthma has not been fully elucidated.

We have previously shown that the nonselective COX inhibitor, indomethacin, can modulate airway cough reflex sensitivity to inhaled capsaicin [10] but the exact role of COX-2 in this sensitivity remains controversial because another study failed to modulate cough reflex sensitivity to inhaled capsaicin in patients with asthma treated with a COX-2 inhibitor, celecoxib for 1 week [11]. We hypothesized that these conflicting findings might be due to differences in affinity for COX-2 enzymes. Accordingly, we decided to use etodolac in the present study as it has greater affinity than celecoxib for COX-2 enzymes than for COX-1 enzymes [13,14]. Our findings clearly show the beneficial effect of a 2-week treatment program with etodolac on cough reflex sensitivity to inhaled capsaicin. Kamei et al [12] recently showed the antitussive effect of NS-398, a newly generated and highly selective COX-2 inhibitor, in guinea pigs, supporting our observation that COX-2 inhibition is capable of attenuating cough reflex sensitivity in asthmatic airways. We are unsure about the exact mechanism behind the modulatory role of COX-2 because we did not measure arachidonic acid metabolites in this study. Kamei et al, however, proposed a particularly interesting theory in this respect, suggesting that the inhibition of substance P release might result in the regulation of endogenous prostaglandins by COX-2 inhibitors on the capsaicin-sensitive sensory C-fibers. It could therefore be speculated that COX-2, generated in chronic eosinophilic inflammation [1,7-9], might modulate airway cough reflex sensitivity in asthmatic airways.

Another major problem in clinical practice is intolerance to nonsteroidal anti-inflammatory drugs (NSAIDs) [23]. Although we did not study aspirin intolerance in our study, none of our patients experienced a wheezing attack. Several studies have demonstrated the safety of highly selective COX-2 inhibitors in patients with aspirin-induced asthma, suggesting that this form of bronchoconstriction is mediated through COX-1-inhibiting pharmacological activity and not through an allergic mechanism [24-29]. Titchen et al [30], however, reported adverse drug reactions caused by several NSAIDs, including a case of severe asthma attack induced by rofecoxib. Their enlightening findings indicate that the safety of COX-2 inhibitors should be corroborated in larger populations and longer-term studies. A number of recent placebo-controlled

trials have produced new evidence about the cardiovascular risks of rofecoxib, celecoxib, and valdecoxib [17,18]. Although a subsequent study did not find etodolac to be associated with elevated cardiovascular risk [31], long-term adverse reactions should be clarified in future studies.

In conclusion, the present study clearly showed that etodolac, a potent COX-2 inhibitor, attenuated cough reflex sensitivity to inhaled capsaicin in the airways of patients with asthma, indicating a possible role by COX-2 in airway cough reflex sensitivity in asthmatic airways with chronic eosinophilic inflammation. Further studies are required to analyze the inflammatory process in such airways following COX-2 induction.

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