CHRONIC OBSTRUCTIVE PULMONARY DISEASE

Effects of cyclo-oxygenase inhibition on exhaled eicosanoids in patients with COPD

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Background: Leukotriene (LT) B_4 concentrations are increased and prostaglandin (PG) E_2 concentrations are decreased in exhaled breath condensate (EBC) in patients with chronic obstructive pulmonary disease (COPD). A study was undertaken to investigate the short term effects of cyclo-oxygenase (COX) inhibition on exhaled LTB_4 and PGE_2 concentrations in patients with COPD and to identify the COX isoform responsible for exhaled PGE_2 production.

Methods: Two studies were performed. A double blind, crossover, randomised, placebo controlled study with ibuprofen (400 mg qid for 2 days), a non-selective COX inhibitor, was undertaken in 14 patients with stable COPD, and an open label study with oral rofecoxib (25 mg once a day for 5 days), a selective COX-2 inhibitor, was undertaken in a different group of 16 COPD patients. EBC was collected before and after drug treatment. Exhaled LTB_4 and PGE_2 concentrations were measured with specific immunoassays.

Results: All patients complied with treatment as indicated by a reduction in ex vivo serum thromboxane B_2 concentrations (ibuprofen) and a reduction in lipopolysaccharide induced increase in ex vivo plasma PGE_2 values (rofecoxib) of more than 80%. Exhaled LTB_4 was increased after ibuprofen (median 175.5 (interquartile range 128.8–231.5) pg/ml *v* 84.0 (70.0–98.5) pg/ml, $p<0.001$) and exhaled PGE_2 was reduced (93.5 (84.0–105–5) pg/ml *v* 22.0 (15.0–25.5) pg/ml, $p<0.0001$). Rofecoxib had no effect on exhaled LTB_4 ($p=0.53$) or PGE_2 ($p=0.23$).

Conclusions: Non-selective COX inhibition decreases PGE_2 and increases LTB_4 in EBC, whereas selective COX-2 inhibition has no effect on these eicosanoids. PGE_2 in EBC is primarily derived from COX-1 activity, and COX inhibition may redirect arachidonic acid metabolism towards the 5-lipoxygenase pathway.

Concentrations of leukotriene (LT) B_4, a potent neutrophil chemoattractant, are detectable in sputum in patients with stable chronic bronchitis[1] and are raised in patients with chronic obstructive pulmonary disease (COPD) during acute exacerbations.[2] Prostaglandin (PG) E_2, an endogenous bronchodilating compound, may have anti-inflammatory effects in the airways.[3–5] The isoprostanes are prostaglandin-like compounds which are formed by the free radical catalysed peroxidation of arachidonic acid in the plasma membrane phospholipids.[6 7]

LTB_4, PGE_2, and 8-isoprostane (15-F_{2t}-isoprostane or 8-iso-$PGF_{2\alpha}$) concentrations are increased in exhaled breath condensate (EBC) in patients with COPD.[8 9] EBC is a completely non-invasive method of sampling secretions from the airways.[10–12] Preliminary data show that ibuprofen, a non-selective cyclo-oxygenase (COX) inhibitor, decreases exhaled PGE_2 concentrations in patients with stable COPD.[13] As PGE_2 may have anti-inflammatory effects in the airways, raised PGE_2 levels in COPD might represent a mechanism for counteracting lung inflammation in this disease.[8] This is further supported by the selective increase in exhaled PGE_2 in patients with COPD but not in asthmatics,[14] and the correlation between exhaled LTB_4 and PGE_2 concentrations in patients with COPD.[8] Taken together, these data indicate that non-selective COX inhibition might have important implications for lung inflammation in COPD patients.

A study was undertaken to investigate the short term effect of non-selective and selective COX-2 inhibition on exhaled LTB_4, PGE_2, and 8-isoprostane concentrations in patients with stable COPD and to identify the COX isoform responsible for exhaled PGE_2 production.

METHODS

Study design

Two studies were performed. The first study was a double blind, crossover, randomised, placebo controlled study with ibuprofen. The study duration was 11 days. Patients attended the outpatient clinic at the Royal Brompton Hospital, London on days 1, 3, 9, and 11. Visit 1 was a screening visit for clinical examination, spirometric tests, skin prick testing, EBC collection, and venous blood sampling. On the same day they were randomised to receive either oral ibuprofen (400 mg every 6 hours) or matched placebo for 2 days. After 2 days of treatment and a morning dose of 400 mg of either ibuprofen or placebo, all the above tests except skin prick tests were repeated (visit 2). After a 5 day washout the patients underwent the other arm of the study.

The second study was an open labelled, uncontrolled study of rofecoxib, a selective COX-2 inhibitor. Patients attended the COPD outpatient clinic of the Department of Internal Medicine, Catholic University of the Sacred Heart, Rome, Italy on three occasions for clinical examination, EBC collection, spirometric tests, sputum induction, measurement of arterial blood gas tensions, and venous blood sampling. After a screening visit (visit 1) and a 2 week run-in period (visit 2), patients were given oral rofecoxib (25 mg once daily

Abbreviations: COPD, chronic obstructive pulmonary disease; COX, cyclo-oxygenase; EBC, exhaled breath condensate; FEV_1, forced expiratory volume in 1 second; FVC, forced vital capacity; LTB_4, leukotriene B_4; NSAID, non-steroidal anti-inflammatory drug; PGE_2, prostaglandin E_2; TxB_2, thromboxane B_2

Table 1 Characteristics of ibuprofen study subjects (n = 14)*

Age (years)	63 (3)
F/M	6/8
Smoking	Ex-smokers
Pack years†	>10
FEV_1 (l)	0.96 (0.11)
FVC (l)	2.06 (0.14)
FEV_1 (% pred)	38.2 (3.8)
FVC (% pred)	65.9 (3.8)
FEV_1/FVC (%)	45.8 (2.8)
Change in FEV_1 post-bronchodilator (% pred)	2.3 (1.1)
Atopy‡	No
Treatment	
β_2 agonists	4
Anticholinergics	6

FEV_1, forced expiratory volume in 1 second; FVC, forced vital capacity.
*Data refer to patients who completed the study and are expressed as mean (SE).
†All the patients had a smoking history of more than 10 pack years.
‡Atopy was an exclusion criterion. Absence of atopy was confirmed by skin prick testing.
Six of the 14 patients were treated with inhaled corticosteroids: beclomethasone dipropionate (dose range 0.2–1 mg/day), budesonide (dose range 0.4–0.8 mg/day).

for 5 days). After treatment all the above tests were repeated (visit 3). Skin prick testing was performed at visit 1.

Day to day repeatability of eicosanoid measurements in both the ibuprofen and rofecoxib study group patients was assessed in a randomised design before patients entered the study by collecting three EBC samples within 7 days of the first.

The ibuprofen study was approved by the ethics committee of the Royal Brompton Hospital and Harefield Trust, London, and the rofecoxib study was approved by the ethics committee of the University Hospital ''A Gemelli'', Catholic University of the Sacred Heart, Rome. Informed consent was obtained from each patient.

Table 2 Characteristics of rofecoxib study subjects (n = 16)*

Age (years)	68 (2)
F/M	6/10
Smoking†	Ex-smokers
Arterial HbCO (%)	0.7 (0.2)¶
Pack years	>10
FEV_1 (l)	1.51 (0.11)
FVC (l)	2.82 (0.18)
FEV_1 (% pred)	59.6 (3.4)
FVC (% pred)	85.8 (3.8)
FEV_1/FVC (%)	54.4 (2.6)
PEF (l/s)	4.25 (0.34)
PEF (% pred)	58.7 (4.2)
$FEF_{25–75}$ (l/s)	0.58 (0.07)
$FEF_{25–75}$ (% pred)	19.4 (2.5)
Change in FEV_1 post-bronchodilator (% pred)	2.2 (1.4)
Atopy‡	No
Treatment	
β_2 agonists	5
Anticholinergics	4

FEV_1, forced expiratory volume in 1 second; FVC, forced vital capacity; HbCO, carbomonoxyhaemoglobin; PEF, peak expiratory flow; $FEF_{25–75}$, mid forced expiratory flow.
*Data refer to patients who completed the study and are expressed as mean (SE).
†All the patients had a smoking history of more than 10 pack years.
‡Atopy was an exclusion criterion. Absence of atopy was confirmed by skin prick testing.
¶All values are <2.6%. One patient was excluded from the study because of recent smoking exposure on visit 3 (HbCO 4.6%) and COPD exacerbation.

Subjects

In the ibuprofen study 15 patients with stable severe COPD were studied (table 1), of whom 14 completed the study. One patient was excluded because of lack of compliance.

In the rofecoxib study 17 patients with stable moderate COPD were enrolled (table 2), 16 of whom completed the study. One patient was excluded because of COPD exacerbation and recent smoking exposure.

In both studies the diagnosis and classification of COPD severity were based on the Global Initiative for Obstructive Lung Disease (GOLD) guidelines.[15] All patients who completed the studies were clinically stable with baseline post-bronchodilator forced expiratory volume in 1 second (FEV_1) <80% predicted and FEV_1/forced vital capacity (FEV_1/FVC) ratio <70% which did not change markedly over 8 weeks. Patients with other respiratory or systemic diseases were excluded from the studies. All patients had negative bronchodilator reversibility testing defined as an increase in FEV_1 that was both lower than 200 ml and 12% above the pre-bronchodilator FEV_1 after inhalation of 400 µg salbutamol. All study subjects were ex-smokers and had stopped smoking for at least 6 months. They all had a negative history for atopic disease and negative skin prick testing. In the ibuprofen study, smoking status was checked by measuring urinary cotinine levels which were <10 ng/ml in all study subjects (data not shown). Six of the 14 patients were treated with inhaled corticosteroids (table 1). Patients had never received LT receptor antagonists or 5-lipoxygenase inhibitors. None of the patients had received non-selective non-steroidal anti-inflammatory drugs (NSAIDs) in the previous 10 days and none had ever received COX-2 inhibitors.

In the rofecoxib study, smoking status was checked by measuring arterial carbomonoxyhaemoglobin (HbCO, table 2). None of the patients had received corticosteroids in the previous 4 weeks. Patients had never received LT receptor antagonists, 5-lipoxygenase inhibitors, or COX-2 inhibitors. As two patients were on treatment with NSAIDs at baseline (visit 1), a 10 day washout period preceded the run-in. Inhaled β_2 adrenergic agonists and anticholinergic drugs were used in some patients in both groups (tables 1 and 2).

Pulmonary function

Spirometric parameters were measured using a dry spirometer (Vitalograph Ltd, Buckingham, UK) in the ibuprofen study and a 10 l bell spirometer (Biomedin, Padova, Italy) in the rofecoxib study. Three to a maximum of six acceptable manoeuvres were performed until the two largest FEV_1 and FVC values were within 0.2 l of each other. The best value was chosen.

Exhaled breath condensate collection and measurement of exhaled eicosanoids

In both studies EBC was collected using a condensing chamber (Ecoscreen, Jaeger, Hoechberg, Germany) as described previously.[9] Subjects were instructed to breath tidally through a mouthpiece connected to the condenser for 15 minutes while wearing a noseclip. An average of 1.0 ml EBC per patient was collected in the ibuprofen study and 1.1 ml EBC in the rofecoxib study. Samples were stored at −80°C before eicosanoid measurements. α-Amylase concentrations in all EBC samples were measured by an in vitro colorimetric method (detection limit 22 U/ml) to check for possible salivary contamination (Roche Diagnostics, Basel, Switzerland).

LTB_4 was measured by a commercially available enzyme immunoassay (Cayman Chemical, Ann Arbor, Michigan,

USA);[8 16] PGE_2 and 8-isoprostane were measured by radioimmunoassays using specific antisera.[17] Specificity for LTB_4, 8-isoprostane, and PGE_2 was previously confirmed by reverse phase-high performance liquid chromatography (RP-HPLC).[16 17] The immunoassay detection limit was 4 pg/ml for LTB_4 and 10 pg/ml for 8-isoprostane and PGE_2. The intra-assay and interassay coefficients of variation for eicosanoid were as follows: LTB_4, <10% and <15%, respectively; 8-isoprostane, <4% and <11%, respectively; PGE_2, <4% and <5%, respectively.

Sputum induction

Sputum induction was performed as previously described.[18] Subjects inhaled 3.5% saline for 15 minutes via an ultrasonic nebuliser (DeVilbiss 2000, DeVilbiss Co, Heston, Middlesex, UK) with a calibrated mass median aerodynamic diameter of 4.5 μm and output of 4.5 ml/min. Secretions collected during the first 5 minutes were discarded to minimise squamous epithelial cell contamination. Samples were considered adequate for analysis if there was <50% squamous cell contamination. Sputum samples were processed immediately. Dithiothreitol solution (0.1%) (Sigma-Aldrich Co, St Louis, MO, USA) was added to the sputum and the mixture was allowed to stand for 30 minutes. Cell viability was determined by exclusion of Trypan Blue. After centrifugation at 1500*g* for 8 minutes, the cell pellet was resuspended in 10 ml Presercyt solution and fixed overnight. Thin layer slides were prepared using the Thin Prep 2000 automated slide processor (Cytyc Co, Boxborough, MA, USA).[19] 100 μl of suspension were added to 50 ml of Preservcyt solution and placed in the automated slide processor. After dispersion, cells were automatically collected on a polycarbonate filter membrane. The thin, evenly dispersed monolayer of cells was then deposited onto the slide in a 20 mm circle. Residual mucus and erythrocytes were removed in the process. For each determination two slides were prepared and stained with either the Papanicolaou or May-Grünwald-Giemsa method. Differential cell counts were expressed as a percentage of lower airway cells (that is, excluding squamous epithelial cells). Differential cell counts for epithelial cells, macrophages, lymphocytes, and neutrophils were performed using Papanicolaou stained slides; differential cell counts for eosinophils were performed using May-Grünwald-Giemsa stained slides.

Serum thromboxane B_2 (TxB_2) measurement

Compliance with ibuprofen treatment was assessed by measuring ex vivo serum TxB_2 concentrations at each visit as treatment with ibuprofen suppresses production of serum TxB_2 in response to endogenously formed thrombin.[20] Blood drawn by a plastic syringe was immediately transferred into glass tubes and allowed to clot at 37°C for 30 minutes. Serum was separated by centrifugation and stored at −20°C until assayed. Serum TxB_2 concentrations were measured by a radioimmunoassay as described previously.[20]

Plasma PGE_2 measurement

Compliance with rofecoxib treatment was assessed by measuring plasma PGE_2 concentrations after ex vivo platelet COX-2 activation with lipopolysaccharide (LPS) and COX-1 inhibition with aspirin.[21] A venous blood sample (5 ml) was drawn and collected in a heparinised tube before (visit 2) and after treatment with rofecoxib (visit 3). One ml aliquots of whole blood samples were incubated at 37°C for 24 hours in tubes containing LPS (10 μg/ml) to activate COX-2 and a low dose of aspirin (10 μg/ml) to selectively suppress platelet COX-1 or tubes containing no LPS to assess basal PGE_2 production derived from COX-2 (control samples). Plasma was separated by centrifugation and kept at −30°C until assayed for PGE_2 as described previously.[21] COX-2 inhibition after rofecoxib was expressed as percentage of decrease in plasma PGE_2 concentrations compared with pretreatment values in LPS containing samples after subtracting basal plasma PGE_2 concentrations (control samples).

Arterial blood gas tensions

Arterial oxygen and carbon dioxide tensions (Pa_{O_2}, Pa_{CO_2}) and pH were measured using an ABL 510 blood/gas analyser (Radiometer, Copenhagen, Denmark).

Sample size

The sample size for both studies was calculated[22] considering LTB_4 concentrations in EBC as the primary outcome. Sample size was estimated to be 15 subjects based on exhaled LTB_4 concentrations, after having considered a standard deviation of 20 pg/ml, a drop out rate of 20%, and identified the minimal difference of biological significance (33.8 pg/ml) with a power of 90% (α value of 5% and β value of 10%). Size effect corresponds to a 33% increase in LTB_4 concentrations after drug treatment.

Statistical analysis

LTB_4, PGE_2, and 8-isoprostane concentrations in EBC and cell counts in sputum were expressed as medians with interquartile range (IQR) in parentheses. Exhaled LTB_4, PGE_2 and 8-isoprostane concentrations, cell counts in sputum within the treatment group, and comparisons before and after treatment were analysed using Friedman's repeated measures of analysis of variance. Spirometry, arterial blood gases, serum TxB_2, and plasma PGE_2 values followed a normal distribution and were expressed as mean (SE). Spirometric values and arterial blood gases within the treatment group and comparisons before and after treatment were analysed using repeated measures of analysis of variance with

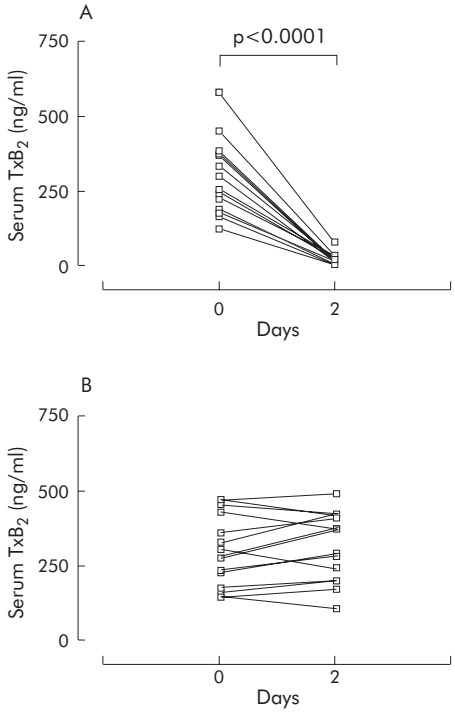

Figure 1 Serum thromboxane B_2 (TxB_2) concentrations ex vivo before (day 0) and after treatment (day 2) with (A) oral ibuprofen (400 mg four times a day) or (B) matched placebo for 2 days in patients with COPD (n = 14). Values are expressed as means.

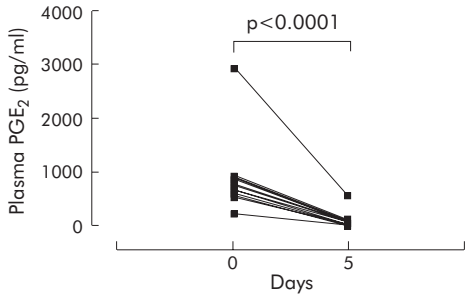

Figure 2 Plasma prostaglandin E_2 (PGE_2) concentrations after ex vivo platelet COX-2 activation with lipopolysaccharide (10 μg/ml) and COX-1 inhibition with aspirin (10 μg/ml) before (day 0) and after treatment (day 5) with oral rofecoxib (25 mg once a day for 5 days) in patients with COPD (n = 16) Values are expressed as means.

Newman-Keuls test. For comparing plasma PGE_2 and serum TxB_2 values, a paired *t* test was used. A p value of <0.05 was considered significant. Correlation was expressed as Spearman's correlation coefficient.

RESULTS

No amylase activity (<22 U/ml) was detected in any study sample, excluding significant salivary contamination.

Mean serum TxB_2 concentrations ex vivo were reduced by 93.9% after treatment with ibuprofen (p<0.0001, fig 1A) but not after placebo (p = 0.29, fig 1B). The reduction in TxB_2 concentration was more than 85% in all patients, indicating compliance with treatment. Following rofecoxib treatment there was a reduction in the LPS induced increase in plasma PGE_2 concentrations compared with pretreatment values (p<0.0001, fig 2). This reduction was more than 80% in all patients, indicating compliance with treatment. Treatment with ibuprofen and rofecoxib was well tolerated.

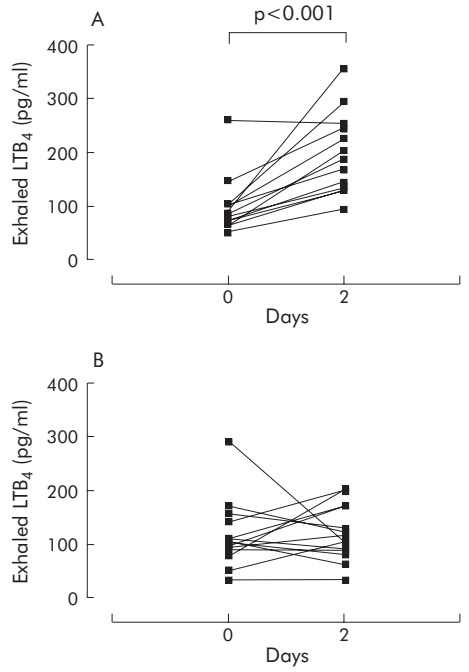

Figure 3 Leukotriene B_4 (LTB_4) concentrations in exhaled breath condensate before (day 0) and after treatment (day 2) with (A) oral ibuprofen (400 mg four times a day) or (B) matched placebo for 2 days in patients with COPD (n = 14). Values are expressed as medians.

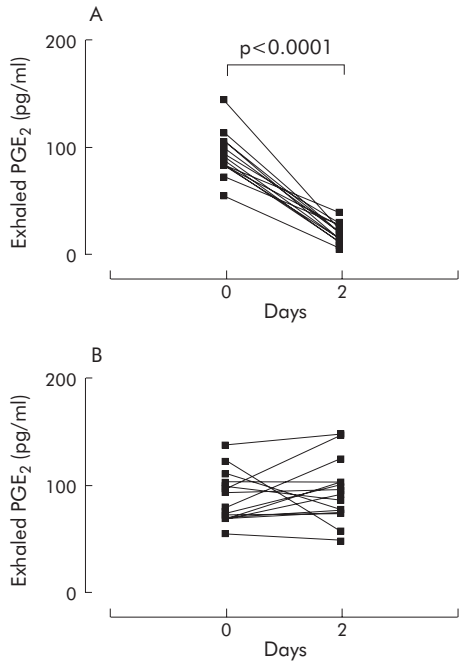

Figure 4 Prostaglandin E_2 (PGE_2) concentrations in exhaled breath condensate before (day 0) and after treatment (day 2) with (A) oral ibuprofen (400 mg four times a day) or (B) matched placebo for 2 days in patients with COPD (n = 14). Values are expressed as medians.

Exhaled breath condensate

Ibuprofen study

Exhaled LTB_4 concentrations were increased after drug treatment (175.5 (128.8–231.5) pg/ml *v* 84.0 (70.0–98.5) pg/ml), p<0.001; fig 3A) but not after placebo (104.5 (92.8–126.5) pg/ml *v* 111.0 (91.0–150.5) pg/ml, p = 0.87; fig 3B). Exhaled PGE_2 concentrations were reduced after treatment with ibuprofen (93.5 (84.0–105.5) pg/ml *v* 22.0 (15.0–25.5) pg/ml, p<0.0001; fig 4A) but not after placebo (88.0 (72.5–103.0) pg/ml *v* 95.5 (72.5–104.0) pg/ml, p = 0.49; fig 4B). Ibuprofen did not affect exhaled 8-isoprostane concentrations (ibuprofen: 47.9 (40.5–51.9) pg/ml pre-treatment *v* 42.2 (34.9–60.0) pg/ml post-treatment, p = 0.64, fig 5A; placebo: 38.3 (31.0–43.8) pg/ml pre-treatment *v* 40.6 (25.1–45.2) pg/ml post-treatment, p = 0.87, fig 5B).

There was no correlation between exhaled eicosanoids and spirometric values. The intraclass correlation coefficient was 0.75 for LTB_4 (p<0.001), 0.87 for PGE_2 (p<0.0001) and 0.79 for 8-isoprostane (p<0.001).

Rofecoxib study

Rofecoxib had no effect on median exhaled LTB_4 (pre-treatment 89.4 (81.8–116.4) pg/ml *v* post-treatment 83.8 (64.4–105.6) pg/ml, p = 0.53; fig 6A), PGE_2 (pre-treatment 89.3 (75.5–100.5) pg/ml *v* post-treatment 91.5 (85.5–102.8) pg/ml, p = 0.23; fig 6B), or 8-isoprostane concentrations (pre-treatment 39.5 (33.0–46.5) pg/ml *v* post-treatment 39.5 (34.8–43.5) pg/ml, p = 0.93; fig 6C).

There was a negative correlation between 8-isoprostane concentrations in EBC and Pao_2 values at baseline ($r = -0.67$, p = 0.045, n = 16) (fig S3 available online at http://www.thoraxjnl.com/supplemental). There was no correlation between exhaled eicosanoids and spirometric values. There was no correlation between exhaled markers and either absolute or relative cell counts in sputum. The intraclass correlation coefficient was 0.67 (p<0.001) for LTB_4, 0.82 (p<0.0001) for PGE_2, and 0.75 (p<0.0001) for 8-isoprostane.

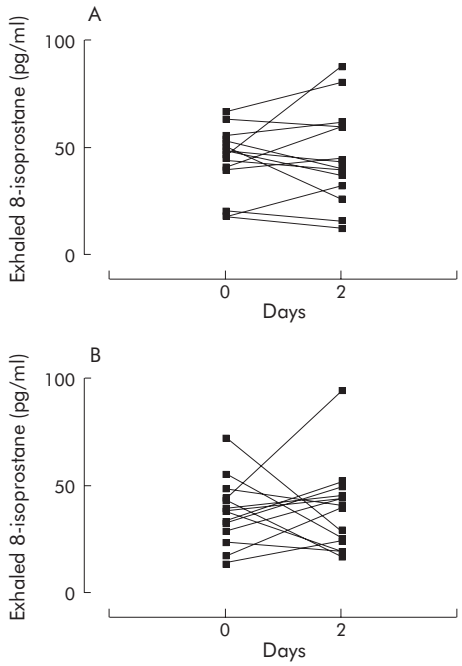

Figure 5 8-Isoprostane concentrations in exhaled breath condensate before (day 0) and after treatment (day 2) with (A) oral ibuprofen (400 mg four times a day) or (B) matched placebo for 2 days in patients with COPD (n = 14). Values are expressed as medians.

Pulmonary function tests

Ibuprofen study

Treatment with ibuprofen or placebo had no effect on FEV_1 ($p = 0.61$), FVC ($p = 0.26$), and FEV_1/FVC ratio ($p = 0.99$) (see tables S1 and S2 available online at http://www.thoraxjnl.com/supplemental).

Rofecoxib study

Rofecoxib had no effect on FEV_1 ($p = 0.45$), FVC ($p = 0.52$), and FEV_1/FVC ratio ($p = 0.82$) (see table S3 available online at http://www.thoraxjnl.com/supplemental).

Sputum induction

Rofecoxib study

Three samples from one patient had more than 50% squamous cell contamination and were excluded from analysis. Differential or absolute counts of any cell type at baseline and after the run-in period were similar (see table S4 available online at http://www.thoraxjnl.com/supplemental).

Rofecoxib had no effect on absolute (total cells, $p = 0.55$; macrophages, $p = 0.77$; neutrophils, $p = 0.77$; lymphocytes, $p = 0.42$; eosinophils, $p = 0.77$) or differential sputum cell counts (squamous cells, $p = 0.77$; macrophages, $p = 0.31$; neutrophils, $p = 0.59$; lymphocytes, $p = 0.53$; eosinophils, $p = 0.69$) (see table S4 available online at http://www.thoraxjnl.com/supplemental).

Arterial blood gases

Rofecoxib study

In 10 out of 16 patients arterial blood gas measurements were performed on visits 1, 2, and 3. Six patients who participated in the study refused to have a second arterial blood gas measurement. In the 10 patients who had three arterial blood gas measurements the mean Pa_{O_2} was increased by 4.2 mm Hg after rofecoxib, although statistical significance was not reached (pre-treatment 73.3 (1.8) mm Hg; post-treatment 77.5 (3.3) mm Hg, $p = 0.42$) (see fig S4A available online at http://www.thoraxjnl.com/supplemental).

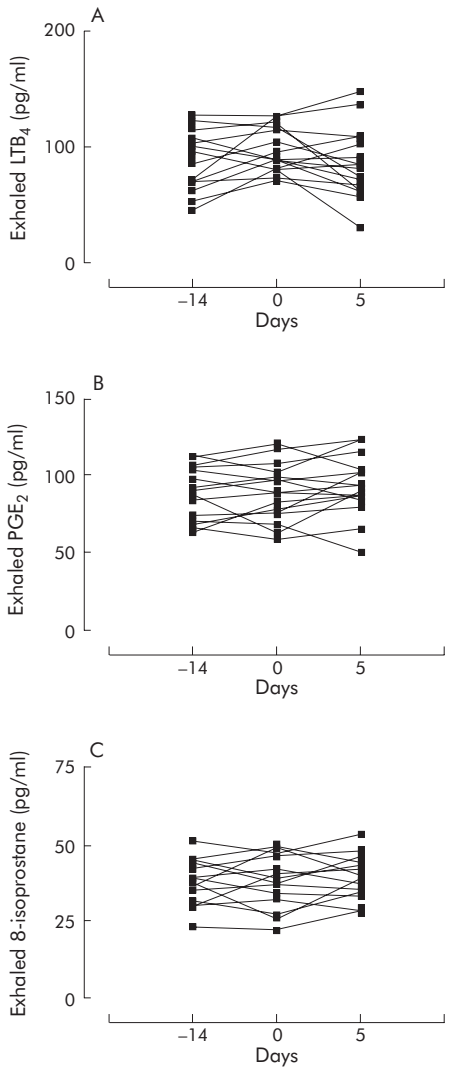

Figure 6 (A) LTB_4, (B) PGE_2, and (C) 8-isoprostane concentrations in exhaled breath condensate in patients with COPD at baseline (day −14), before (day 0), and after treatment with oral rofecoxib 25 mg once a day for 5 days (day 5) in patients with COPD (n = 16). Values are expressed as medians.

Rofecoxib had no effect on Pa_{CO_2} or pH values (see fig S4B and C in online supplement).

DISCUSSION

We have investigated the effects of COX inhibition on exhaled LTB_4, PGE_2, and 8-isoprostane concentrations in patients with COPD. All patients complied with treatment as reflected by a reduction in ex vivo serum TxB_2 concentrations after ibuprofen of more than 85% and inhibition of LPS induced increase in plasma PGE_2 values after rofecoxib of more than 80% in each patient. The specificity of the enzyme immunoassay for LTB_4 and the radioimmunoassays for 8-isoprostane and PGE_2 has been shown previously by RP-HPLC.[16 17] Day to day repeatability for exhaled eicosanoid measurements is acceptable, as indicated by their intraclass correlation coefficients consistent with previous findings.[23] In a randomised, double blind, placebo controlled, crossover study, ibuprofen, a non-selective COX inhibitor, given orally at a dose of 1600 mg/day for 2 days, increased LTB_4 concentrations and decreased PGE_2 concentrations in EBC in patients with stable COPD. By contrast, in a different study of open label design, oral administration of rofecoxib

(25 mg/day for 5 days), a selective COX-2 inhibitor, had no effect on these eicosanoids. Other authors have reported increased LTB_4 concentrations in bronchoalveolar lavage (BAL) fluid from allergic COX-1 deficient mice compared with allergic wild type and COX-2 deficient mice.[24] Taken together, these data indicate that COX-1 inhibition can divert arachidonic acid to other metabolic products (such as leukotrienes) under conditions of increased lung inflammation. LTB_4 is a potent chemoattractant for neutrophils which have an important pathophysiological role in COPD.[25] Increased exhaled LTB_4 concentrations in patients with COPD treated with ibuprofen might indicate that airway inflammation in these patients is increased after non-selective COX inhibition. These effects are not observed after rofecoxib, which indicates that selective COX-2 inhibitors might be more tolerated than other NSAIDs in patients with COPD. However, the lack of administration of a non-selective and a selective COX-2 inhibitor to the same group of patients and the open label uncontrolled design of the rofecoxib study limit the strength of these findings and preclude definitive conclusions.

In the ibuprofen study six of the 14 patients were on inhaled corticosteroids, whereas all the rofecoxib study group patients were steroid naïve. A previous observational study showed that inhaled steroids had a limited, if any, effect on exhaled eicosanoids in patients with COPD.[8] However, studies are needed to address formally the influence of steroid treatment on exhaled eicosanoids in patients treated with COX inhibitors. As ibuprofen decreases exhaled PGE_2 concentrations in patients with COPD while rofecoxib does not, the present study indicates that COX-1 is primarily responsible for exhaled PGE_2 production. Similar PGE_2 levels in BAL fluid were reported in allergic wild type and COX-2 deficient mice.[24] By contrast, prostaglandin synthesis in allergic guinea pig lungs in vitro has been reported to be significantly dependent on COX-2.[26] This discrepancy may partially be explained by in vitro/in vivo differences in expression and regulation of COX isoforms[24] and/or differences in the inflammatory process (allergen challenge *v* COPD). Data on the effect of COX inhibitors in the present studies should be interpreted cautiously as COPD severity was different in the rofecoxib and ibuprofen study group patients (moderate and severe, respectively). Further studies are required to clarify whether the effect of COX inhibitors on exhaled eicosanoids in COPD is affected by disease severity, whether the difference between the action of the two drugs is related to COX independent mechanisms, and the long term effect of COX inhibitors on exhaled eicosanoids in patients with COPD. Findings on the functional consequences of COX inhibition on airway inflammation are controversial,[26 27] possibly due to the fact that COX inhibitors abrogate the synthesis of both pro- and anti-inflammatory prostaglandins (PGD_2 *v* PGE_2).[28] The lack of effect of ibuprofen and rofecoxib on exhaled 8-isoprostane concentrations indicates that this compound is primarily formed independently of the COX pathway.[7]

The values of exhaled eicosanoids in our study are given per ml EBC, as in most of the published studies on exhaled biomarkers. This approach may have limitations since many factors are likely to affect differentially the volume of EBC and the concentrations of various compounds in it, including differences in EBC collection, sample storage, analytical techniques, and disease severity.[11] As pointed out by Effros and co-workers, part of the variation in the concentrations of non-volatile compounds in EBC can be related to differences in the dilution of respiratory droplets by water vapour.[29] The lack of correlation between structurally related compounds such as 8-isoprostane and PGE_2 observed in this study and the selective increase of leukotrienes (LTB_4 *v* LTE_4) in the single subject previously reported[8] do not seem to support this evidence. However, reference indicators, such as the measurement of conductivity as proposed by Effros and co-workers,[30] should be used in future studies aimed at quantifying exhaled eicosanoids. At present, because of the lack of a standardised procedure for EBC collection and validated analytical methods, comparisons of data obtained in different laboratories are difficult.[11] The fact that eicosanoid concentrations in EBC are not more diluted than those observed in BAL fluid might indicate that these compounds are somewhat volatile and reach the EBC in the gas phase. However, recent data indicate that LTB_4 is not volatile (P Montuschi, unpublished data). The physical and chemical properties of eicosanoids in EBC are largely unknown and require further research. EBC analysis is currently more reliable for measuring relative values than for determining absolute levels of inflammatory mediators.

No changes were seen in lung function tests or sputum cell counts after short term treatment with oral ibuprofen or rofecoxib at therapeutic doses. Similarly, 1 week of treatment with oral celecoxib (200 mg twice daily), another selective COX-2 inhibitor, did not affect bronchial responsiveness in asthmatic patients.[31] However, possible effects on lung function and/or sputum cell counts after longer treatment with COX inhibitors cannot be ruled out. The mean Pao_2 was increased by 4.2 mm Hg after rofecoxib, although statistical significance was not reached ($p = 0.42$). The biological significance of these findings is currently unknown, as is the effect on Pao_2 of long term treatment with selective COX-2 inhibitors.

In conclusion, short term non-selective COX inhibition increases LTB_4 and reduces PGE_2 concentrations in EBC in patients with COPD, whereas selective COX-2 inhibition does not seem to have these effects. These findings indicate that exhaled PGE_2 is primarily derived from COX-1 activity and that COX-1 inhibition may redirect arachidonic acid metabolism towards the 5-lipoxygenase pathway. However, controlled studies with non-selective and selective COX inhibitors in the same group of patients with COPD are required to draw definitive conclusions.

Figures S1–S4 and tables S1–S4 are available in the online supplement at http://www.thoraxjnl.com/supplemental

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LUNG ALERT

Rapid disease progression is an important cause of death in IPF

▲ Martinez FJ, Safrin S, Weycker D, *et al* for the IPF Study Group. The clinical course of patients with idiopathic pulmonary fibrosis. *Ann Intern Med* 2005;**142**:963–8

The authors examined data generated from the placebo arm of a large multicentre double blind trial of interferon-γ1b in patients with mild to moderate idiopathic pulmonary fibrosis (IPF), collected over a median period of 76 weeks at 12 weekly intervals.

IPF was diagnosed according to the ATS criteria, by surgical lung biopsy or established HRCT criteria in 168 patients (mean age 64 years, 66% male, 86% white, 91% never or ex-smokers). At entry most patients (82%) were receiving oral corticosteroids in a dose of less than 15 mg daily, the mean duration since diagnosis was over 1 year, and the mean (SD) carbon monoxide transfer factor (TLCO) was 36.8 (10.6)%. Patients who survived to week 72 (78.6%) had minimal deterioration in physiology and dyspnoea (FVC, TLCO, A–a gradient, transitional dyspnoea index). Thirty six patients (21.4%) died during the observation period. In those who died of an IPF related death (32/36), nearly 50% died acutely (<4 weeks). Progression of IPF and pneumonia were the most common causes (63% and 13%, respectively). Patients who died had a progressive decline in measures of physiology and dyspnoea throughout the observation period. More than one third of patients were admitted to hospital, often frequently (1.67 admissions/patient), 23% for a respiratory disorder with a mean length of stay of 14.3 days.

This study provides novel insights into the natural history of IPF. In particular, a large percentage of deaths had an acute onset, suggesting that accelerated IPF may be more common than was once perceived. Hospitalisation occurred commonly and frequently. A substantial proportion of patients had a minimal decline in conventional measures of lung function and dyspnoea, highlighting disease heterogeneity and posing challenges for measuring efficacy of new pharmaceutical compounds. Further prospective studies are required to elaborate on these findings.

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