

ORIGINAL ARTICLE

Effects of celecoxib on major prostaglandins in asthma

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Summary

Background Prostaglandin (PG) D₂ is a pro-inflammatory and bronchoconstrictive mediator released from mast cells, and is currently evaluated as a new target for treatment of asthma and rhinitis. It is not known which cyclooxygenase (COX) isoenzyme catalyses its biosynthesis in subjects with asthma.

Objectives Primarily, to assess whether treatment with the COX-2 selective inhibitor celecoxib inhibited biosynthesis of PGD₂, monitored as urinary excretion of its major tetranor metabolite (PGDM). Secondly, to determine the effects of the treatment on biosynthesis of PGE₂, thromboxane A₂ and PGI₂, also measured as major urinary metabolites.

Methods Eighteen subjects with asthma participated in a cross-over study where celecoxib 200 mg or placebo were given b.i.d. on 3 consecutive days following 2 untreated baseline days. Six healthy controls received active treatment with the same protocol. Urinary excretion of the eicosanoid metabolites was determined by liquid chromatography/tandem mass spectrometry (LC/MS/MS). Lung function was followed as FEV₁ and airway inflammation as fraction of exhaled nitric oxide (F_ENO).

Results Celecoxib treatment inhibited urinary excretion of PGEM by 50% or more in subjects with asthma and healthy controls, whereas there was no significant change in the excretion of PGDM. In comparison with the healthy controls, the subjects with asthma had higher baseline levels of urinary PGDM but not of PGEM. The 3-day treatment did not cause significant changes in FEV₁ or F_ENO.

Conclusion and Clinical Relevance Biosynthesis of PGD₂ was increased in subjects with asthma and its formation is catalysed predominantly by COX-1. By contrast, COX-2 contributes substantially to the biosynthesis of PGE₂. The asymmetric impact of celecoxib on prostanoid formation raises the possibility of long-term adverse consequences of COX-2 inhibition on airway homeostasis by the decreased formation of bronchodilator PGs and maintained production of increased levels of bronchoconstrictor PGs in asthmatics.

Keywords Asthma, Celecoxib, NSAIDs, salivary prostaglandin E₂, urinary prostaglandin metabolites

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Introduction

Prostaglandins (PG) are important mediators in inflammation as reflected by the clinical efficacy of common non-steroidal anti-inflammatory drugs (NSAIDs) which inhibit their biosynthesis [1]. The discovery of two cyclooxygenase (COX) iso-enzymes that catalyse the oxygenation of arachidonic acid into the endoperoxides PGG₂ and PGH₂ [2, 3] provided new opportunities to define the biology of the COX pathway by the use of iso-

enzyme selective inhibitors [4]. Accordingly, biosynthesis of the potent platelet aggregator and vasoconstrictor thromboxane (TX) A₂ largely derives from COX-1 [4], whereas *in vivo* formation of the vasodilating inhibitor of platelet aggregation, prostacyclin (PGI₂) is inhibited substantially by selective COX-2 inhibitors [5]. The undesirable cardiovascular side-effects of the selective COX-2 inhibitors, the coxibs, can largely be attributed to the suppression of PGI₂ [6].

The role of PGs in asthma and airway inflammation, however, remains unclear. Although inhalation of PGE₂ inhibits bronchoconstriction induced by several factors that trigger asthma attacks [7], the reports on the effects of NSAIDs in bronchoprovocation studies of asthmatic subjects are conflicting [8, 9, 10]. Moreover, bronchoconstriction induced by the intake of aspirin and all other non-selective NSAIDs is the defining characteristic of the peculiar syndrome of aspirin-intolerant asthma (AIA) [11].

Whereas PGE₂ may be a bronchoprotective mediator, two other COX products, TXA₂ and PGD₂, are potent bronchoconstrictors in humans [12, 13]. In addition, PGD₂ has a number of pro-inflammatory actions [14], especially in mouse models [15]. The relative abundance of PGD₂ and PGE₂ may thus be an important factor in determining the impact of NSAIDs and aspirin in asthma. However, it is not known if the biosynthesis of PGD₂ in subjects with asthma is catalysed by COX-1 or COX-2. In mouse mast cells *in vitro*, the early-phase of PGD₂ production is COX-1 dependent, whereas induction of COX-2 has been associated with a delayed, but more prolonged production of PGD₂ [16]. *In vivo* data from mouse models suggest that COX-2 may control PGD₂ biosynthesis after induction of airway inflammation [15, 17], whereas at baseline, biosynthesis derives largely from COX-1 [18].

The current study was initiated to establish the effects of the selective COX-2 inhibitor, celecoxib, on the biosynthesis of eicosanoids, as reflected by major urinary metabolites of PGD₂ and PGE₂, in subjects with asthma. In addition to measurements of PGDM and PGEM, liquid chromatography/tandem mass spectrometry (LC/MS/MS) determination of major urinary metabolites of TXA₂ (TXM) and PGI₂ (PGIM) was also performed [19]. This was performed to determine whether the overall effects of the intervention on these COX products was similar in asthmatics as observed previously in healthy subjects or patients with other chronic diseases [18, 19].

Although controlled trials have shown that subjects with the syndrome of AIA tolerate coxibs [20, 21, 22], there is no published report on the effects of coxibs on PG biosynthesis or airway function in the general population of subjects with asthma. To define the pathway for formation of PGD₂ in asthma is of specific relevance to understanding why subjects with AIA are sensitive to isoform non-specific NSAIDs, but still tolerate COX-2 inhibitors. It has been established that a bronchoconstrictor adverse reaction to NSAIDs in these patients paradoxically is associated with an increase in the release of PGD₂ [23]. Thus, the tolerance of COX-2 inhibitors by such patients might be attributable to inhibition of PGD₂ biosynthesis.

The asthmatic subjects in this study were recruited to include three different phenotypes; intermittent asthma (IA), aspirin-tolerant asthma (ATA) and AIA. The idea

behind this design was to gather hypothesis generating data on whether or not there were major qualitative differences in pathways for PG biosynthesis between these three subgroups.

A recent study of leukotriene biomarkers in asthma found significant differences in the levels of salivary leukotrienes between distinct phenotypes of asthma [24]. Salivary PGE₂ is increased in patients with various diseases [25], and its use as a non-invasive biomarker of systemic inflammation has been proposed. There is, however, no information on salivary PGE₂ levels in subjects with asthma, nor whether the COX-1 or COX-2 pathway catalyses its formation in salivary glands *in vivo*. In this study, we therefore tested the hypothesis that salivary PGE₂ in the subjects with asthma is sensitive to treatment with celecoxib.

Methods

Subjects

The Ethical Review Board in Stockholm approved the study (Dnr 2007/865-31), and the subjects gave written informed consent. Eighteen subjects aged 18–65 years with diagnosed asthma were recruited from the Lung and Allergy Clinic at Karolinska University Hospital. The study group comprised six subjects with IA, six subjects with persistent ATA and 6 subjects with AIA. Subject characteristics are displayed in Table 1. Subjects with AIA had chronic asthma, recurrent rhinosinusitis and/or nasal polyps and intolerance to NSAIDs verified by a positive lysine-ASA inhalation challenge [11]. Subjects with ATA had similar afflictions of the upper and lower airways as the AIA group, but tolerated aspirin. All patients were in a stable condition with a forced expiratory volume in 1 s (FEV₁) of 70% of predicted or more. They had not had a respiratory tract infection or suffered exacerbations in the 6 weeks before the study. They had never smoked or were non-smokers for the last 2 years with less than a 5-packyear lifetime smoking history.

Specific exclusion criteria were hypersensitivity to sulphonamides or COX-2 inhibitors, a history of serious gastrointestinal adverse events, inflammatory bowel diseases, a tendency to fluid retention or being at risk to that, impaired hepatic or renal function, all NYHA-classes of heart failure, established cardiovascular disease or risk factors for such diseases and other ischaemic vascular disease (except well-controlled hypertension) and current use of ASA/NSAID or any other anti-inflammatory drugs [apart from inhaled corticosteroids (ICS)] for the 2 weeks preceding the study. Patients treated with oral glucocorticosteroids or inhaled steroids at higher doses than 1200 µg budesonide or equivalent and those on current medications that would interact with celecoxib were also excluded. Subjects with respiratory diseases other than

Table 1. Asthmatic subjects' characteristics

Subject identity	Gender	Age (year)	FEV ₁ (litre)	FEV ₁ % predicted	ICS (µg)	Positive SPT number	F _E NO (p.p.b.)
AIA							
A001	F	24	4.0	117	160	–	62
A002	M	50	3.2	82	1200	–	47
A003	M	39	3.7	89	320	–	14
A016	F	43	2.2	84	320	1	19
A019	M	57	2.3	68	1200	–	26
A020	F	41	2.2	73	320	–	15
ATA							
A005	M	30	3.3	97	320	4	8
A006	M	28	3.1	79	720	5	11
A007	F	23	2.5	88	320	2	97
A008	F	23	3.0	87	400	–	12
A010	F	27	3.2	101	400	1	21
A015	F	45	2.8	108	320	5	25
IA							
A009	F	51	2.7	97	–	4	13
A011	M	29	4.5	106	–	4	62
A012	M	33	4.5	113	–	1	48
A013	M	23	4.7	100	–	3	34
A014	M	25	4.7	109	–	3	14
A018	F	23	3.8	101	–	3	11

AIA, aspirin-intolerant asthma; ATA, aspirin-tolerant asthma; IA, intermittent asthma; FEV₁, forced expiratory volume in 1 s; ICS, inhaled corticosteroid expressed as budesonide equivalent; SPT; skin prick test; F_ENO, fraction of exhaled nitric oxide; M, male; F, female.

asthma, or co-morbidities that could affect the end-points in the study were excluded.

Study design

The study was randomized cross-over and single-blind with a 2-week washout between the two treatment periods. Each period consisted of five clinic visits between 8 and 10 a.m. on consecutive weekdays, Monday to Friday, and with baseline pre-treatment measurements in the mornings of the first 2 study days (Monday and Tuesday). The study drug, celecoxib 200 mg b.i.d. (Celebra[®]; Pfizer, Sollentuna, Sweden), or placebo b.i.d. as administered as capsules (active drug and placebo capsules purchased from the hospital pharmacy) on study days 2–5 during each period, with the first dose taken immediately after obtaining baseline measurements on day 2. This level of dosing of celecoxib has been established previously to cause profound inhibition (>75%) of monocyte-derived COX-2-catalysed formation of PGE₂ in whole blood *ex vivo* with a minimal (<25%) reduction in COX-1-derived TXB₂ from platelets [5].

All subjects underwent a screening visit when informed consent was obtained, and medical history, physical examination and spirometry was performed. Blood was sampled for routine haematology and blood chemistry to ensure normal liver and renal function. For safety reasons, subjects with AIA came for an extra visit when two doses of celecoxib, each of 100 mg, were given 1 h apart

followed by 2 h observation. This extra visit was followed by a 1-week washout before the study proper commenced.

A group of six healthy individuals were also recruited to provide a control group for the effects of celecoxib on urinary PG. The volunteers participated in the celecoxib study period but did not receive placebo.

Visit procedures included measurements of FEV₁ using a spirometer (Jaeger Masterscope, Intramedic AB, Bålsta, Sweden), exhaled NO (NIOX[™], Aerocrine AB, Stockholm, Sweden) and collection of urine and saliva.

Collection of urine and saliva

The first morning urine sample was voided at home and one sample was collected at the unit during the visit. The voided volume was measured and the urine samples were stored at –70 °C until assayed. The subjects were informed not to take any food or beverage, except water, within 1.5 h before sampling. They were then instructed to wash their mouth with water before collecting 5 mL of whole saliva into a plastic tube. The sample was stored at –70 °C. Before assay, the sample was thawed and centrifuged at 1500×*g* for 10 min (+4 °C), and the supernatant was subsequently analysed.

Measurements of metabolites

The urinary levels of eicosanoid metabolites were determined by tandem LS/MS/MS technology as described [18,

19], and expressed as ng/mg creatinine. PGD₂ was monitored as 11,15-dioxo-9-hydroxy-2,3,4,5-tetranorpropane-1,20-dioic acid (PGDM), PGE₂ as 11 α -hydroxy-9,15-dioxo-2,3,4,5-tetranor-prostane-1,20-dioic acid (PGEM), PGI₂ as 2,3-dinor-6-keto-PGF_{1 α} (PGIM) and TXA₂ as 11-dehydro-TXB₂ (TXM). Internal standards were d₆- PGDM (Cayman Chemical Company, Ann Arbor, MI, USA; catalog no. 10009039), d₆- PGEM (Cayman 314840), d₃-2,3-dinor-6-keto PGF_{1 α} (Biomol BML-PG040-0050, Plymouth Meeting, PA, USA) and d₄-11-dehydro-TXB₂ (Cayman 319500). The assay robustness was described in previous publication [18, 19]. Lower limits of detection were obtained by calculating the signal-to-noise ratio (s/n) for each of the four analytes from five random samples, then extrapolating to an s/n of 3. This is expressed as the average ($\pm$ SD). PGEM: 11 ($\pm$ 5) pg; PGDM: 23 ($\pm$ 13) pg; d6k: 16 ($\pm$ 5) pg; and 11deH: 23 ($\pm$ 7) pg.

Measurement of PGE₂ in saliva was performed with an enzyme immunoassay (EIA) from Cayman and expressed as pg/mL of saliva. The detection limit was 15 pg/mL. The antibody had <0.01% cross-reactivity with other primary PG of the two-series and their immediate metabolites, but 43% cross-reactivity with PGE₃, 20% with PGE₁ and 1–3% for isoprostanes E₂ and F₂, respectively.

The investigators that determined prostanoid levels by MS or EIA were blinded to the study code at the time of analysis.

Statistical analysis

All statistical analysis in this study was carried out using the Sigma Stat statistical software package (Aspire Software Int., Ashburn, VA, USA). The effects of treatments on urinary metabolites were analysed using the Wilcoxon signed-ranks test for the cross-over periods. For between-group comparisons the Mann–Whitney rank sum test or Kruskal–Wallis one way analysis of variance on ranks were used as appropriate. For lung function, the paired *t*-test was used. A *P*-value of <0.05 was accepted as being statistically significant. Data are displayed as median and 25th–75th percentiles unless otherwise indicated.

Results

Urinary prostanoid metabolites in subjects with asthma

In the 18 subjects with asthma, the levels of the urinary metabolites on 3 consecutive days of treatment with celecoxib or placebo were compared with 2 preceding baseline days during each period. The baseline values (mean of day 1 and day 2) of any of the metabolites of PGD₂, PGE₂, PGI₂ and TXB₂, were not different between the two treatment periods (Figs 1a, c, e, g, Table 2).

Celecoxib did not affect urinary PGDM concentrations with mean levels during treatment (days 3–5), expressed

either as raw values or in percent of baseline, being no different from that of placebo (*P*<0.05) (Figs 1a and b, Table 2). In contrast, levels of urinary PGEM decreased significantly during treatment with celecoxib (Fig. 1c, Table 2). The median [25th–75th percentiles] percent inhibition of PGEM during the 3-day treatment with celecoxib was 45.1 [32.8–57.2] (*P*<0.01 compared with the placebo). The effect of celecoxib was progressive with 56.2 [44.5–74.0] percent inhibition on day 5 (Fig. 1d) compared with 47.5 [17.6–58.2] (*P*<0.05) on the first day of treatment. The excretion of urinary PGIM was also significantly decreased after celecoxib (Fig. 1e, Table 2). The median percent inhibition of PGIM was 36.6 [13.3–62.6] on days 3–5 (*P*<0.05 compared with the placebo), and 46.6 [33.3–68.6] on day 5 (Fig. 1f). Excretion of the established COX-1 product, TXA₂, was not affected by celecoxib (Figs 1g and h, Table 2).

Among the subjects with asthma, the levels of PGEM were significantly higher in males compared with females (9.3 [6.2–19.6] vs. 4.9 [3.4–7.3] ng/mg creatinine; *P*<0.05), whereas the levels of the three other COX metabolites displayed no gender differences (data not shown). The effect of celecoxib on PGEM levels was, however, not different between the gender groups. The median percent of baseline [25th–75th percentiles] levels on day 5 was 42.0 [19.7–55.0] and 43.8 [39.8–55.9] for males and females, respectively.

Urinary excretion of PGD and PGE metabolites in healthy volunteers

In the healthy control group (*n* = 5–6), the findings were similar with no significant changes of urinary PGDM levels after 3 days of treatment with celecoxib (median [25th–75th percentiles] days 3–5 vs. baseline: 0.66 [0.36–0.72] and 0.86 [0.65–0.87] ng/mg creatinine, respectively; *P*>0.05), but reduced PGEM excretion (days 3–5 vs. baseline: 2.2 [1.62–2.42] and 6.74 [2.61–10.91], respectively; *P*<0.05). The percent inhibition of PGEM over the three treatment days was 56.5 [28.8–77.4], and on day 5 the inhibition was 75.0 [39.4–85.9] percent.

Subgroup analyses of urinary metabolites

There were no significant differences in urinary excretion of any of the studied metabolites between those with IA, persistent ATA or AIA, neither with respect to the effect of the COX-2 inhibition nor on baseline levels of the measured urinary metabolites.

In comparison with the healthy controls, the asthmatic subjects had significantly higher baseline concentrations of PGDM (2.46 [1.66–3.19] and 0.78 [0.66–0.85] ng/mg creatinine in asthmatic subjects and in healthy volunteers, respectively; *P*<0.001), whereas the PGEM levels were

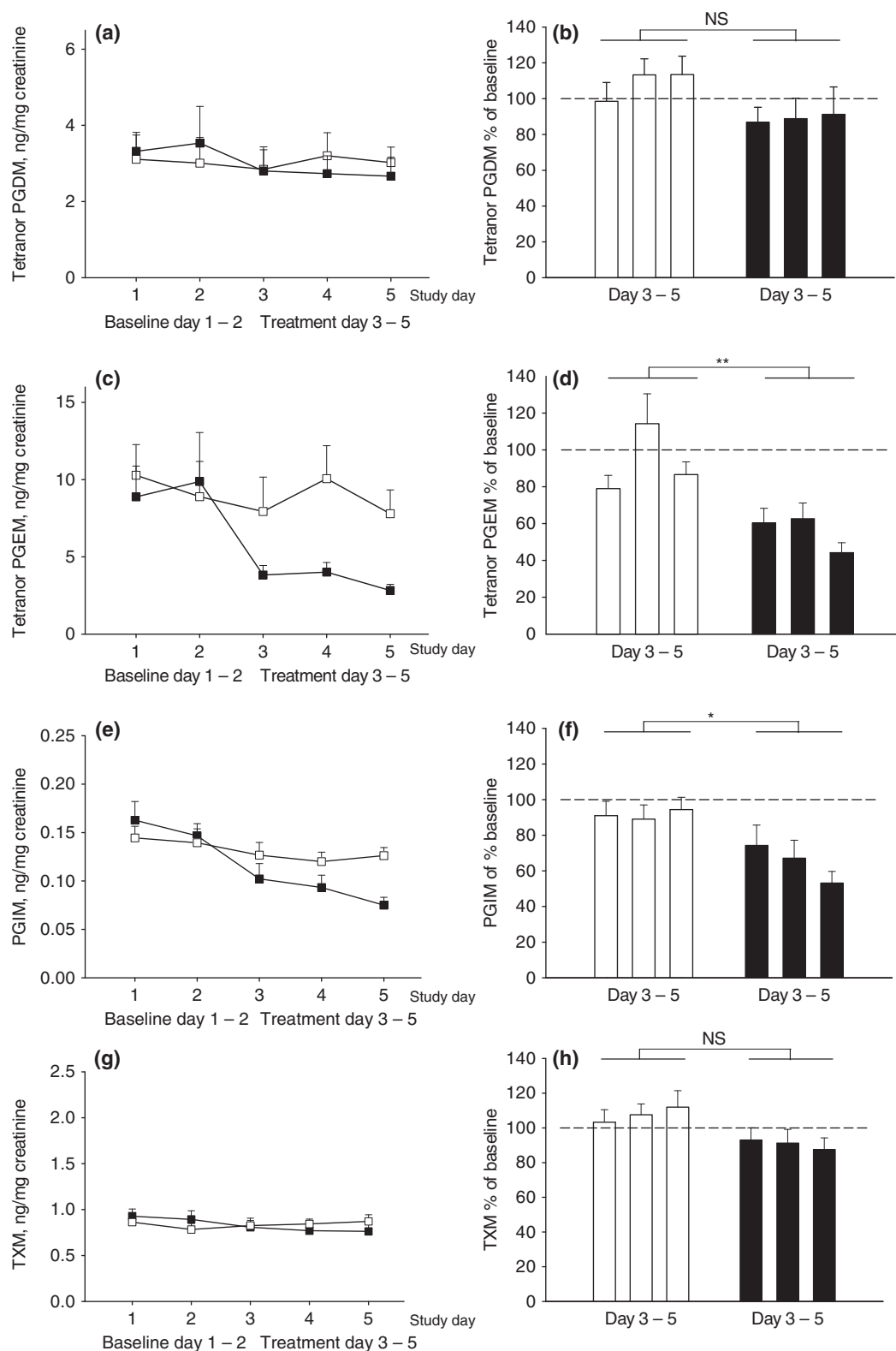


Fig. 1. Mean (SEM) urinary concentrations of (a) PGDM, (c) PGEM, (e) PGIM and (g) TXM determined by LC/MS/MS (liquid chromatography-tandem mass spectrometry), during 2 untreated baseline days and on 3 consecutive days after daily intake of placebo (open square) or the selective COX-2 inhibitor, celecoxib, 200 mg b.i.d. (filled square). The design was single blind, cross-over with 2 weeks washout in 18 subjects with asthma. (b, d, f, h) Levels of the respective metabolite expressed as percent of baseline (mean of study days 1 and 2, dotted line in figures indicate baseline level). PGDM and TXM were not affected by the treatment, whereas PGEM and PGIM decreased significantly ($P < 0.001$ and $P < 0.05$, respectively). * $P < 0.05$ compared with placebo. ** $P < 0.01$ compared with placebo.

Table 2. Concentrations of prostaglandins in urine and saliva from 18 subjects with asthma after a 3-day cross-over treatment with celecoxib (200 mg b.i.d.) and placebo

Prostaglandin metabolite	Placebo		Celecoxib	
	Baseline	Treatment	Baseline	Treatment
Urinary PGDM	2.2 [1.7–2.8]	2.3 [1.8–3.5]	2.4 [1.6–3.8]	1.9 [1.2–3.8]*
Urinary PGEM	7.9 [4.2–10.2]	6.0 [4.8–9.4]	4.5 [3.8–9.9]	3.2 [1.9–4.6]*†
Urinary PGIM	0.13 [0.1–0.19]	0.14 [0.1–0.14]	0.14 [0.11–0.21]	0.08 [0.06–0.12]*†
Urinary TXM	0.73 [0.68–0.94]	0.81 [0.68–0.93]	0.83 [0.63–1.13]	0.78 [0.6–0.86]
Salivary PGE ₂	107 [69–180]	99 [63–139]	103 [67–129]	76 [56–129]*†

Levels are expressed as group median [25th–75th percentiles] at baseline (mean of days 1–2) and during treatment (mean of days 3–5).

* $P < 0.05$ compared with baseline.

† $P < 0.05$ compared with placebo.

PGDM, PGEM, PGIM and TXM in ng/mg creatinine, PGE₂ in pg/mL.

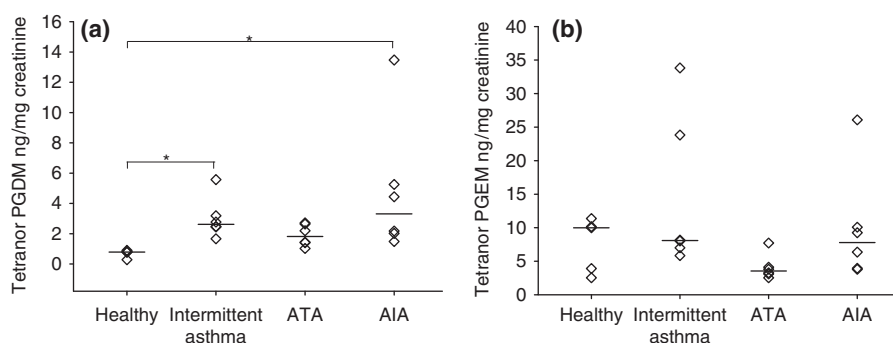


Fig. 2. Baseline urinary concentrations of (a) PGDM and (b) PGEM in healthy volunteers ($n = 5$) and in three groups of asthmatic subjects; intermittent asthma (IA) ($n = 6$), aspirin-tolerant asthma ($n = 6$) and aspirin-intolerant asthma ($n = 6$). The asthmatic subjects as a whole ($n = 18$) displayed significantly higher levels of PGDM compared with healthy volunteers ($P < 0.001$), as did the two subgroups of IA and AIA. Open diamonds indicate individual levels and horizontal lines indicate median value for each group. * $P < 0.05$.

not significantly different between the groups (Figs 2a and b).

There was a weak but significant negative correlation between the dose of ICS and the inhibition of PGEM excretion within the group of asthmatics, such that the larger the dose of ICS, the smaller was the effect of celecoxib on PGE₂ levels ($r = 0.48$, $P = 0.044$). This correlation was not seen for the inhibition of PGI₂ (data not shown).

Salivary PGE₂

The levels of PGE₂ in saliva were the same on the 2 baseline days of the two periods ($P > 0.05$) (Fig. 3a, Table 2). During treatment with celecoxib, there was a small but significant decrease in the level of PGE₂, whereas there was no change after placebo (Fig. 3b, Table 2). The effect of celecoxib corresponded to a median 30.3 [–7.8 to 48.3] percent inhibition, which, however, did not reach statistical significance compared with the placebo ($P > 0.05$). There were no gender differences in salivary PGE₂ levels. The median [25th–75th percentiles] levels for all 5 days of

placebo treatment were 106 [58–154] and 107 [78–135] in males and females, respectively.

Lung function

There were no significant changes in FEV₁ or F_ENO during 3 days of treatment with celecoxib or placebo. Mean (SEM) FEV₁ percent predicted days 3–5 was 96.9 (2.6) and 96.1 [2.6] with placebo and celecoxib, respectively, and median [25th–75th percentiles] F_ENO days 3–5 was 16.0 [11.2–30.0] and 23.0 [12.0–53.6] p.p.b., respectively.

Discussion

Measurements of the stable urinary prostanoid metabolites has documented that biosynthesis of PGD₂ in subjects with asthma is maintained during 3 days of treatment with celecoxib. Biosynthesis of TXA₂, primarily derived from COX-1 [4, 6] is also unaffected by the COX-2 inhibitor, celecoxib. In contrast, the treatment caused a profound inhibition of biosynthesis of PGE₂ and PGI₂, each known to be generated substantially by COX-2 activity [5, 26].

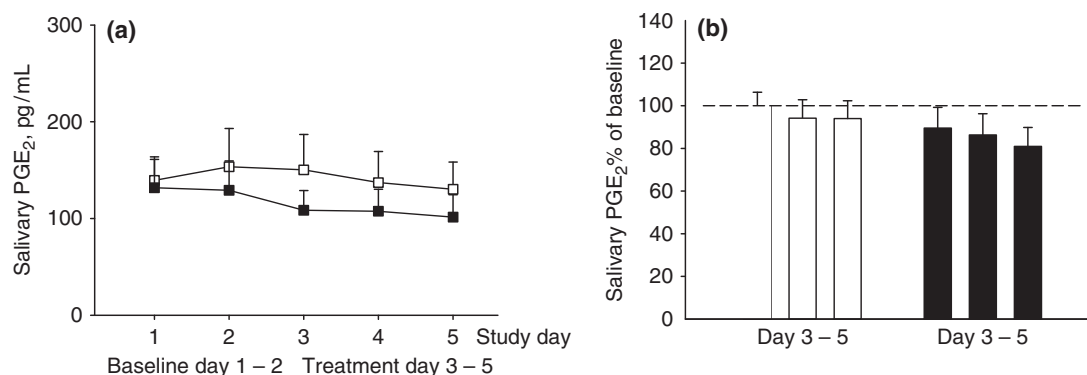


Fig. 3. Concentrations of PGE₂ in saliva determined by enzymeimmunoassay, during 2 untreated baseline days and on 3 consecutive days after daily intake of placebo (open square) or the selective COX-2 inhibitor, celecoxib, 200 mg b.i.d. (filled square). (a) Mean (SEM) daily levels (b) percent of baseline. The mean inhibition with celecoxib was 19% after 3 days of treatment.

The study therefore provides new evidence that basal biosynthesis of PGD₂ in asthmatics, as described previously in healthy volunteers [18], is generated predominantly by COX-1. The study also documents that the levels of the metabolites of PGD₂ in urine are high, about three to five times higher than those of TXA₂ and PGI₂, and in a similar range as for metabolites of PGE₂.

The study examined the influence of celecoxib on urinary PGDM and PGEM in a group of healthy control subjects and found the same effects of the intervention as in the asthmatics. Our data agree with previous studies of the effects of celecoxib and other coxibs on urinary excretion of the major metabolites of PGE₂, TXA₂, PGI₂ and PGD₂ in healthy subjects and patients with cardiovascular diseases [5, 18, 19, 26], as well as the only previous investigation where urinary excretion of PGE metabolites has been measured in subjects with asthma after a single dose of celecoxib [27]. The latter study did not measure other prostanoids. We did not assess the biosynthesis of PGF_{2α}, as analysis of this compound is confounded by the presence of free radical catalysed isoprostanes [28]. Taken together, it is concluded that COX-1 is the quantitatively dominant pathway for *in vivo* biosynthesis of PGD₂, and this would presumably apply to the mast cell that is thought to be the major source of PGD₂ in humans [29]. Notably, our *in vivo* finding of the COX-1-driven biosynthesis of PGD₂ differs from results obtained with cultured human and murine mast cells where COX-2 also may contribute [16, 30].

One particular strength of this study is that for the first time it measures four major prostanoids at baseline and during the intervention with celecoxib. Previous studies in subjects with asthma, including our own, have measured only one family of compounds, e.g. metabolites of PGD₂ or PGE₂. With respect to the implications of the findings for mechanisms in asthma, one limitation with the study may seem to be that it measures whole body biosynthesis rather than local formation in the airways. It might thus

be speculated that pulmonary mast cells release PGD₂ in reactions that are initiated by activation of COX-2, but that this is not reflected when total body production is monitored as urinary excretion of PGDM. However, the lung is well perfused and the clearance of locally formed metabolites into the systemic circulation is efficient. Indeed, biosynthetic rates of prostanoids *in vivo* at baseline are very low relative to the capacity of cells or tissues to form these compounds when activated *ex vivo* or *in vitro* [31]. This means that even minor and transient increases in systemic availability of prostanoids is reflected by the detectable increases in excretion of urinary metabolites. Accordingly, a number of bronchoconstrictive responses mediated by mast cell activation are promptly reflected by increased urinary excretion of metabolites of PGD₂ [23, 32, 33, 34] and TXA₂ [9, 35]. Moreover, inhalation of sodium cromoglycate, which inhibits local mast cell activation in the airways, is associated with significantly decreased urinary excretion of PGD₂ metabolites [34, 36]. It is therefore likely that measurement of urinary PGDM does reflect local biosynthesis in the lung and airways. The higher levels in asthma that we observed provide circumstantial support for this argument. It has also been reported that lung cancer is associated with higher levels of urinary PGEM [26], further supporting that urine is a suitable matrix for monitoring events in the lung.

As the asthma symptoms of the patients in our study were controlled by treatment, it might be argued that COX-2 contributes to PGD₂ formation when induced in mast cells during exacerbations and other states with an increased systemic or local inflammation. Nevertheless, our finding that the PGDM level in healthy volunteers was lower than in the asthmatic patients, may reflect an increased mast cell activation even at baseline in asthma. This is consistent with reports of increased numbers of mast cells in the smooth muscle of subjects with asthma [37]. The similar effect of celecoxib in healthy controls

and asthmatic subjects does not suggest that asthmatic airway inflammation is associated with deviations in the pathway for biosynthesis of PGD₂.

Although the 3-day treatment caused more than 50% reduction in the biosynthesis of the bronchoprotective PGE₂, it was neither associated with any significant changes in baseline lung function nor exhaled NO as a surrogate marker of airway inflammation [38]. However, the purpose of this short-term study was to define the metabolic pathways for baseline formation of PGE₂ and PGD₂ in subjects with asthma. Longer treatment periods and assessment of the effects of coxib treatment on bronchial responsiveness to allergen and other trigger factors in asthma are required to define the safety profile of COX-2 inhibitors in asthma.

The study included a group of subjects with well-characterized AIA. The finding that they tolerated celecoxib adds to previous indications [20, 21, 22, 27] that COX-2 inhibition does not provoke bronchoconstriction in this particular group of patients that responds with bronchoconstriction to all unselective NSAIDs. Our results also confirmed previous indications that the basal levels of PGE metabolites in urine of intolerant subjects are the same as in aspirin-tolerant subjects with asthma [27]. The investigation provides further mechanistic insight. As formation of PGD₂ was unaltered after celecoxib, the tolerance of COX-2 inhibitors in AIA is not explained by the inhibition of PGD₂. The study also confirmed previous data that basal levels of urinary PGD₂ metabolites in subjects with AIA and ATA are no different [23, 24, 39, 40, 41]. The currently most popular hypothesis to explain NSAID intolerance holds that these particular patients are dependent upon PGE₂ to stabilize their mast cells [11]. The paradoxical release of PGD₂ during the intolerance reaction [23] is thought to be an objective sign of mast cell activation occurring as a result of removal of such protective PGE₂. That mast cell activation occurs is also supported by the release of tryptase during the reaction [11]. As the celecoxib treatment in our study caused more than 50% reduction in PGE₂ levels but did not trigger bronchoconstriction nor PGD₂ release, one interpretation of the current data would be to reject the PGE₂ hypothesis.

The subjects with IA were not treated with ICS, whereas the two other groups were maintained on ICS treatment. This permitted us to assess whether or not the effect of celecoxib was different in two similar-sized groups receiving ICS or not. It was observed that the average influence of celecoxib on PGEM was smaller in subjects treated with inhaled steroids. This would be consistent with the down-regulation of COX-2 by the steroid treatment [2, 3], tilting biosynthesis in the steroid-treated subjects towards COX-1. The difference was, however, small, but is actually consistent with the relatively limited effects of oral steroid treatment on

systemic PG formation in healthy subjects [42]. It will be of interest for future studies to test if steroid treatment alters responsiveness to COX-2 inhibitors when airway inflammation is induced.

As saliva is a convenient non-invasive matrix to collect, and used to monitor biomarkers in different disease states [25], we wanted to test the hypothesis that salivary PGE₂ formation was COX-2 dependent and might be monitored by a simple routine EIA. However, only a minor component of the salivary levels of PGE₂ were accounted for by a celecoxib sensitive pathway. There was no gender difference in salivary PGE₂, whereas the data confirmed previous observations of an increased urinary excretion of metabolites of PGE₂, but no other measured COX metabolite, in males [26, 43, 44].

The reason for or consequence of the higher levels of urinary PGE metabolites in males, observed here and in previous studies, is not clear. As the metabolites measured in this study predominantly are formed in the liver, biosynthesis in the prostate or other accessory genital glands of males is unlikely to explain the gender difference. Previous studies have shown that post-menopausal females have levels that become comparable with males [44], indicating hormonal regulation of systemic production of PGE₂.

Clinical relevance

We report that basal biosynthesis of PGD₂ in humans with asthma is increased and catalysed by COX-1, whereas whole body formation of PGE₂ predominantly is COX-2 dependent. Future long-term studies are warranted to determine if there are clinically important consequences of the asymmetric inhibition of prostanoid biosynthesis that we observed during this short-term treatment with celecoxib, i.e. reduction of the bronchodilator and anti-inflammatory mediator PGE₂ with maintained production of high levels of the bronchoconstrictor and pro-inflammatory mediator PGD₂.

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