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Prostaglandin E₂ systemic production in asthma patients with and without aspirin hypersensitivity.

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ABSTRACT

Rationale: Special regulatory role of prostaglandin E₂ has been postulated in aspirin-induced asthma.

Objectives: To investigate the effects of aspirin on systemic production of prostaglandin E₂ and cysteinyl leukotrienes in patients with asthma.

Methods: We determined urinary concentrations of: 1) two main prostaglandin E₂ metabolites, i.e.:13,14-dihydro-15keto-PGE₂ using commercial enzyme immunoassay and 9,15-dioxo-11 α -hydroxy-2,3,4,5-tetranor-prostane-1,20-dioic acid using gas chromatography/mass spectrometry and 2) leukotriene E₄ using immunoassay. Determinations were performed at baseline and following oral aspirin and celecoxib challenges, in two well-defined asthma phenotypes: aspirin-sensitive and aspirin-tolerant patients.

Measurements and Main Results: Aspirin precipitated bronchial reactions in all aspirin-sensitive, but in none of the aspirin-tolerant patients. Celecoxib 400 mg was well tolerated by all patients except for one with aspirin-induced asthma. At baseline mean prostaglandin E₂ metabolites values did not differ between the groups. Following different aspirin provocation doses, the two main prostaglandin E₂ metabolites were decreased in the aspirin-tolerant group, but their mean level remained unchanged in the aspirin-sensitive group. The dose of aspirin had no effect on the magnitude of the response on the prostaglandin E₂ metabolites and its duration. In both groups urinary prostaglandin E₂ metabolites decreased following celecoxib challenge. No correlation was found between prostaglandin E₂ metabolites and leukotriene E₄.

Conclusions: Aspirin-precipitated asthmatic attacks are not associated with changes in the systemic prostaglandin E₂ production. In contrast, prostaglandin E₂ systemic production becomes depressed by aspirin in non-sensitive patients. This different response might indicate COX-1 dependent prostaglandin E₂ control of inflammatory cells in AIA. Thus, PGE₂ is released during the clinical reactions to aspirin through an alternate COX-2 pathway. Clinical implications of this finding are in line with current observations of good tolerance of the selective COX-2 inhibitors in sensitive patients.

Key words: Aspirin-induced asthma, aspirin-tolerant asthma, eicosanoids, cyclooxygenase, lipoxygenase.

List of abbreviations:

AIA – aspirin-induced asthma

ATA - aspirin-tolerant asthma

PGE₂ – prostaglandin E₂

EP1-4 – prostaglandin receptor 1,2,3,4

PGE₂-M – 3,14–dihydro-15keto-PGE₂

Tetranor-PGE-M -9,15-dioxo-11 α -hydroxy-2,3,4,5–tetranor-prostan-1,20-dioic acid

PGD₂ – prostaglandin D₂

LTE₄ – leukotriene E₄

Cys-LTs – cysteinyl leukotrienes

COX- cyclooxygenase

NSAIDs – nonsteroidal anti-inflammatory drugs

INTRODUCTION

Prostaglandin E₂ (PGE₂) is a bioactive compound formed by actions of cyclooxygenase (COX) and specific PGE synthases.[1] In human airways PGE₂ is produced by many cells, including epithelium, smooth muscle, alveolar cells, macrophages, phagocytes and lymphocytes.[1,2] *In vitro* PGE₂ relaxes smooth muscle and displays a number of inhibitory effects on mast cells degranulation, synthesis of leukotriene B₄, activation of granulocytes and T cells.[3,4,5] PGE₂ elicits a myriad of biological effects acting through four receptors: EP1, EP2, EP3, and EP4. [6,7,8] The response of target cells to PGE₂ varies according to the spectrum of receptors they express.

PGE₂ might be of special importance in aspirin-induced asthma. This is a distinct clinical syndrome affecting 5–10 % adult asthmatics.[9,10] Asthma attacks triggered by aspirin and other nonsteroidal anti-inflammatory drugs (NSAIDs) are associated with inhibition of cyclooxygenase,[11,12] specifically COX–1, but not COX–2, and are characterized by overproduction of cysteinyl-leukotrienes (cys-LTs).[13,14,15] Inhaled PGE₂ protects against both aspirin-precipitated attacks of asthma and the massive release of urinary LTE₄. [16,17] The protection does not seem to be achieved through relaxant effects on bronchial smooth muscle, but rather by suppression of the mediators released from the proinflammatory cells. The incriminated cells could be mast cells or eosinophils. PGE₂ greatly inhibits oxygen bursts in granulocytes, including eosinophils [4] and slows down cys-LTs biosynthesis in peripheral blood mononuclear cells.[18]

Because of the rapid metabolism of prostaglandins E during sampling and isolation of plasma,[1] measurement of stable metabolites in urine rather than parent compound is the most efficacious method of assessing their endogenous production.[19] Moreover, human kidneys are abundant source of prostanoids, including PGE₂. Local biosynthesis of PGE₂, dependent on both constitutive COX-1 and inducible COX-2 in the kidneys varies with age and the salt intake. It is also distinctly affected by several drugs, including angiotensin converting enzyme inhibitors. [20] PGE₂ produced in the kidneys is excreted as a parent compound, and does not affect the pool of metabolites produced by systemic inactivation of PGE₂. [21] The main urinary metabolites of PGE₂ levels are believed to reflect global PGE₂ production. The predominant pathway of PGE₂ metabolism has been shown to involve transformation of PGE₂ by the 15-OH PGDH enzyme to 15-keto-PGE₂. Subsequently 15-keto-PGE₂ is rapidly converted to its main metabolite of PGE₂ (13,14-dihydro-15keto-PGE₂; PGE₂-M) by the enzyme 15-keto-prostaglandin- Δ^{13} reductase. [22] Oxidation process of 13,14-dihydro-15keto-PGE₂ leads to formation of a major metabolite of PGE₂ (9,15-dioxo-11 α -hydroxy-2,3,4,5-tetranor-prostan-1,20-dioic acid; tetranor-PGE-M). Tetranor-PGE-M is a stable urinary metabolite of PGE₁ and PGE₂, and is used as a urinary marker of PGE₂ biosynthesis. [23,24]

We studied urinary excretion of two major PGE₂ metabolites, reflecting systemic production of this prostaglandin. The studies were carried out in aspirin-sensitive and aspirin-tolerant asthmatics, both at baseline and after the aspirin and celecoxib challenge. We expected to distinguish aspirin-intolerance by decrease of systemic PGE₂ production following aspirin challenge. We also looked at the possible relationship between release of PGE₂ metabolites and that of cys-LTs. To our knowledge, we were the first to perform such investigations.

MATERIAL AND METHODS

Subjects

The study population consisted of 19 asthmatic patients sensitive to aspirin (AIA) and 21 asthmatics who tolerated aspirin well (ATA). The patients' characteristics are presented in table 1.

Table 1. Clinical characteristics of the patients.

	AIA (n=19)	ATA (n=21)	P
Age (y)	42.4±13.3 41 (31-53)	43.6±12.5 43 (36-53)	N.S. (0.613)
Female/Male	11/8	13/8	N.S. (0.967)
Duration of asthma (y)	7.8±7.5 5 (1-11)	10.6±9.4 7 (3-18)	N.S. (0.328)
Inhaled steroids yes/no	11/8	17/4	N.S. (0.170)
Inhaled steroids (mcg/d)	1690±712 1600 (1000-2000)	1424±821 1000 (800-1600)	N.S. (0.204)
FEV ₁ baseline (%predicted) placebo day	90.4±10.7 94.8 (82.5-98.1)	90.5±12.1 91.4 (81.5-93.6)	N.S. (0.683)
FEV ₁ baseline (%predicted) aspirin day	91.6±10.8 93.0 (87.7-98.4)	94.1±12.0 94.0 (81.9-100.2)	N.S. (0.708)
FEV ₁ baseline (%predicted) celecoxib day	92.4±12.4 90.9 (85.7-98.6)	90.0±13.8 86.6 (78.0-95.7)	N.S. (0.631)
Total IgE (IU/mL)	182.3±229.8 121 (45.4-226)	206.0±241.6.2 91.9 (39.9-345)	N.S. (0.844)
Skin prick test (n) positive/negative	8/7	9/9	N.S. (0.849)
Blood eosinophil count	425.4±328.4 371 (159-669)	356.7±246.7 296(1164-502)	N.S. (0.667)

Values are expressed as mean±SD, and median (25% and 75% percentiles).

AIA – aspirin-induced asthma

ATA – aspirin-tolerant asthma

N.S. – not significant

The diagnosis of aspirin intolerance was confirmed by oral aspirin provocation tests, performed during the 24 months preceding the study. All ATA patients occasionally used aspirin without any adverse reactions. The patients had stable asthma and their baseline FEV₁ was > 70% of the predicted value on the study day. None had experienced an exacerbation or a respiratory tract infection in the 6 weeks preceding the study. The subjects were instructed to withhold medications that decrease bronchial responsiveness prior to aspirin/celecoxib challenge. Short-acting β_2 -

agonists were not used 8 hours before the challenge. Long-acting β_2 -agonists and theophylline were withdrawn for 24 hours. Short-acting antihistamines and cromones were stopped 5 days before the challenge. Inhaled steroids were allowed at a dose $\leq$ 2000 μ g budesonide per day. None of the patients were treated with systemic corticosteroids or leukotriene modifying drugs.

Basal urinary 13,14-dihydro-15keto-PGE₂ (PGE₂-M) and 9,15-dioxo-11 α -hydroxy-2,3,4,5-tetranor-prostane-1,20-dioic acid (tetranor-PGE-M), and LTE₄ levels were measured in 30 healthy subjects. The controls tolerated NSAIDs well and had no history of adverse reactions to aspirin and other aspirin-like drugs.

The patients gave informed consent and the study was approved by the University Ethics Committee.

Study design

The study consisted of two phases.

In the first phase, at one week intervals, the patients underwent aspirin and celecoxib testing. The single-blind, placebo-controlled oral challenge test with aspirin was carried out on two consecutive days. On the first day four capsules of placebo were administered every 1.5 hour. On the second day the patients were challenged with increasing doses of 27, 44, 117, 312 mg of aspirin, at 1.5 hour intervals, up to the cumulative dose of 500 mg. [25]

On the eighth day a single dose of 400 mg of celecoxib was administered.

In the second phase of the study, following a two-week run-in period, the same aspirin-tolerant asthmatic patients again underwent provocation tests with aspirin at a cumulative dose of 188 mg, i.e. the dose which precipitated asthma attacks in 9 aspirin-intolerant patients. The purpose of the second phase was to exclude a dose dependence of the results obtained in the first phase in ATA group. In the first phase all ATA patients were given aspirin dose of 500 mg while provocation doses of aspirin among AIA patients differed. A single-blind, placebo-controlled oral challenge test with aspirin was carried out on two consecutive days. On the first day three capsules of placebo were administered every 1.5 hours. On the second day the patients were challenged with increasing doses of 27, 44, 117 mg of aspirin, at 1.5 hour intervals, up to the cumulative dose of 188 mg.

Placebo, aspirin, and celecoxib had an identical appearance. The challenge procedure with aspirin and/or celecoxib was interrupted, if a bronchospastic reaction occurred (FEV₁ dropped $\geq$ 20%), or if the maximum cumulative dose of aspirin, and a single dose of celecoxib was reached. The cumulative dose of aspirin causing a 20% fall in FEV₁ was calculated and recorded as PD₂₀ (provocation dose of aspirin).

FEV₁ and extrabronchial symptoms were recorded at baseline, before the challenge tests, and then every 30 minutes until 6 hours after the last dose of aspirin and celecoxib.

In patients with positive aspirin challenge (AIA), urine samples were collected for 13,14-dihydro-15keto-PGE₂ (PGE₂-M) and 9,15-dioxo-11 α -hydroxy-2,3,4,5-tetranor-prostan-1,20-dioic acid (tetranor-PGE-M), and LTE₄ estimations at baseline, at the time of appearance of the bronchial symptoms (time 0), and then 2 and 4 hours later. In ATA patients, whose aspirin challenge was negative, urine samples were collected at baseline, 1.5 hours after the last aspirin dose, i.e. when the cumulative doses of 500 mg (first phase) and 188 mg (second phase) were reached (time 0), and then 2 and 4 hours later.

In case of a single dose of celecoxib, urine samples were collected in the same manner as for the aspirin provocation challenge in ATA and AIA patients.

Lung function

Pulmonary function tests were performed on a flow-integrating computerized pneumotachograph (Pneumoscreen, E. Jaeger, Germany).

Biochemical assays

Urinary PGE₂-M and LTE₄

Urinary 13,14-dihydro-15keto-PGE₂ (PGE₂-M); (Cayman Chemical, Prostaglandin E metabolite EIA Kit) and LTE₄ (Cayman Chemical, Ann Arbor, MI) were measured in unpurified urine samples by direct enzyme immunoassay. [26] Measurements were made at the same time, in duplicates, using the same batch of the reagents. Results of 13,14-dihydro-15keto-PGE₂ (PGE₂-M) and LTE₄ were expressed in picograms per mg of creatinine.

Quantification of urinary tetranor-PGE-M

Urinary concentration of 9,15-dioxo-11 α -hydroxy-2,3,4,5-tetranor-prostane-1,20-dioic acid (tetranor-PGE-M) was measured by gas chromatography/mass spectrometry. [27,28] See methods in the online data supplement.

Statistical analysis

Summary statistics were expressed as mean (M), standard deviation (SD), median (Me) and 25% and 75% percentiles. General Linear Model (GLM) including repeated measures analysis of variance, which takes into account the fact that the outcome measurements are repeated over time within subject was used for multiple comparisons. Logarithmic transformation was used when needed as variance stabilizing transformation. To better describe the changes in time for log transformed data 95% confidence interval (CI) in fold difference units [29] were constructed. Correlation between variables was estimated with the Spearman rank order correlations. A p-value ≤ 0.05 was considered statistically significant.

RESULTS

Clinical reactions

There was no statistical difference in clinical characteristics between the patients with aspirin-induced asthma (positive aspirin challenge test), and those who tolerated aspirin well (negative aspirin challenge test), table 1.

None of the patients developed symptoms after administration of placebo.

In aspirin-sensitive asthmatics bronchial reactions developed after 27 mg in 1 subject, after 44 mg in 1 subjects, after 117 mg in 9 subjects, and following 500 mg in 6. The mean cumulative dose of aspirin was 188 mg. One patient, after receiving aspirin at a dose of 177 mg developed asthma and anaphylactic shock. In another patient abdominal pain accompanied by transient increase in urinary (6718 U/L; normal range 32-640 U/L) and serum (2031 U/mL; normal range 30-110 U/mL) amylase and serum lipase (615 U/L; normal range 23-300 U/L) was recorded.

None of ATA patients developed any clinical symptoms following aspirin or celecoxib challenges. In 1 out of 17 AIA patients, the celecoxib challenge produced dyspnea, and spirometric values (FEV₁) dropped by 21%. This was the same patient who developed shock after aspirin administration. The case was described elsewhere. [30] The remaining 16 AIA patients tolerated celecoxib very well.

All the symptoms were relieved by short-acting β_2 -agonists. Systemic corticosteroids were required in 3 cases only. Adrenaline s.c, was administered to the patient who developed shock.

Urinary 13,14-dihydro-15keto-PGE₂ (PGE₂-M)

The first phase

At baseline (table 2) urinary levels of PGE₂-M did not differ significantly between the study groups, and healthy control subjects (ANOVA, $p=0.52$).

Table 2. Baseline values of eicosanoids in AIA and ATA patients and healthy controls (values represent means of three estimations performed in each patient one week apart).

	AIA (n=19)	ATA (n=21)	Healthy (n=30)	p- value		
				AIA vs ATA	AIA vs HC	ATA vs HC
Urinary PGE ₂ -M (pg/mg creatinine)	477.1±277.69 464 (281-600)	480.7±222.1 451 (342-538)	879.47±210.78 403.5 (303-763)	0.95	0.88	0.72
Urinary tetranor-PGEM (ng/mg creatinine)	11.05±9.64 7.2 (5.4-11.1)	10.27±7.83 7.9 (5.0-12.4)	10.06±8.43 7.6(4.9-10.5)	0.79	0.96	0.87
Urinary LTE ₄ (pg/mg creatinine)	1846.6±2747.4 1347 (355-2036)	342.0±277.7 220 (160-361)	257.0±180.2 222 (119-315)	<0.001	<0.001	0.75

Values are expressed as mean±SD, and median (25% and 75% percentiles).

AIA – aspirin-induced asthma

ATA – aspirin-tolerant asthma

HC - healthy control subjects

After placebo administration, no significant differences in urinary PGE₂-M levels were found between both study groups (ANOVA, $p=0.58$). Urinary PGE₂-M levels increased two hours ($p=0.01$; 95%CI [1.030:1.722 baseline]) following placebo administration, as compared to baseline values, only in AIA patients, fig.1A.

PGE₂-M values on the day of aspirin challenge differed significantly between AIA and ATA patients (ANOVA, $p<0.001$). The levels did not change at any time of the observation period in AIA patients. In ATA group urinary PGE₂-M concentrations decreased two ($p<0.001$; 95%CI [0.417:0.688 baseline]), and four hours ($p<0.001$; 95%CI [0.339:0.559 baseline]) following aspirin challenge tests, as compared to baseline values. They reached the lowest level 4 hours after aspirin test administration, fig 1B.

In case of celecoxib challenge, urinary concentrations of PGE₂-M were significantly higher in aspirin-induced patients (ANOVA, $p=0.04$). In both study groups (AIA and ATA; respectively) urinary PGE₂-M levels decreased at two ($p=0.08$ and $p=0.05$; 95%CI [0.557:0.918 baseline]; respectively), and four hours ($p=0.04$; 95% CI [0.532:0.889] baseline] and $p=0.007$; 95%CI [0.521:0.857 baseline]; respectively) following celecoxib challenge tests, as compared to baseline values, fig 1C.

The dose of aspirin had no effect on magnitude of the response of PGE₂-M and its duration.

At baseline and 4 hours following aspirin challenge, urinary PGE₂-M levels were elevated only in aspirin-intolerant patients with the highest levels of the total serum IgE (Spearman $r=0.6$; $p=0.03$).

Baseline concentrations of the PGE₂-M in urine of healthy controls were 1.59-fold (59%) greater in men than in women ($p=0.01$). In contrast, there was no relationship between gender and urinary PGE₂-M levels at baseline or following any of the challenges in the patients groups studied (ANOVA, $p=0.18$).

The second phase

At baseline urinary levels of PGE₂-M did not differ significantly between placebo and aspirin days ($p=0.38$) in ATA patients.

After placebo administration, no significant differences in urinary PGE₂-M levels were found. Urinary PGE₂-M levels decreased two ($p=0.005$; 95%CI [0.488;0.737 baseline]) and four ($p<0.001$; 95%CI [0.399;0.603 baseline]) hours following the cumulative dose of 188 mg of aspirin, as compared to baseline values in ATA patients, fig. 2A.

Urinary 9,15-dioxo-11 α -hydroxy-2,3,4,5-tetranor-prostane-1,20-dioic acid (tetranor-PGE-M)

The first phase

At baseline (table 2) urinary levels of tetranor-PGE-M did not differ significantly between both study groups, and healthy control subjects (ANOVA, $p=0.94$).

Following placebo administration, no significant differences in urinary tetranor-PGE-M levels were found between AIA and ATA patients (ANOVA, $p=0.55$). The levels did not change at any time of the observation period (ANOVA, $p=0.99$), fig. 3A.

Tetranor-PGE-M values following aspirin challenge did not change at any time of the observation period in AIA patients (ANOVA, $p=0.23$), but in ATA patients they decreased significantly (ANOVA, $p<0.001$). In ATA group urinary tetranor-PGE-M concentrations decreased at two ($p=0.001$; 95%CI [0.489;0.748 baseline]), and four hours ($p<0.001$; 95%CI [0.391;0.615 baseline]) following aspirin challenge tests, as compared to baseline values, fig. 3B.

In case of celecoxib challenge, no significant differences in urinary tetranor-PGE-M levels were found between AIA and ATA patients (ANOVA, $p=0.58$). In both study groups (AIA and ATA; respectively) urinary tetranor-PGE-M levels decreased at two ($p<0.001$; 95%CI [0.586;0.933 baseline] and $p<0.001$; 95%CI [0.565;0.888 baseline]; respectively), and four ($p=0.009$; 95%CI [0.352;0.878 baseline]; and $p<0.001$; 95%CI [0.483;0.759 baseline]; respectively) hours following celecoxib challenge, as compared to baseline values, fig. 3C.

The dose of aspirin had no effect on magnitude of the response of tetranor-PGE-M and its duration (ANCOVA, $p=0.32$). The concentration of urinary tetranor-PGE-M in

the individual AIA patients did not depend on the cumulative dose of aspirin which caused bronchospasm.

Baseline concentrations of the tetranor-PGE-M in urine from healthy controls were 1.39-fold (39%) greater in men than in women ($p=0.03$). In contrast, there was no relationship between gender and urinary tetranor-PGE-M levels at baseline or following any of the challenges in the patients groups studied (ANOVA, $p=0.26$).

The second phase

At baseline urinary levels of tetranor-PGE-M did not differ significantly between placebo and aspirin days ($p=0.51$) in ATA patients.

After placebo administration, no significant differences in urinary tetranor-PGE-M levels were found. Urinary tetranor-PGE-M levels decreased two ($p<0.001$; 95%CI [0.558:0.962 baseline]) and four ($p<0.001$; 95%CI [0.466:0.887 baseline]) hours following the cumulative dose of 188 mg of aspirin, as compared to baseline values in ATA patients, fig. 2B.

Urinary LTE_4

Baseline urinary LTE_4 excretion (table 2) was higher in patients with aspirin-induced asthma than in ATA patients (ANOVA, $p<0.001$) or healthy control subjects.

In AIA group following placebo administration the gradual decrease of the urinary LTE_4 level was observed, reaching in the time "0" $p<0.001$; 95%CI [0.317;0.751 baseline], in the subsequent moments of observation (2. and 4. hours) still falling down (each $p<0.001$; 95%CI respectively [0.237;0.561 baseline] and [0.166;0.394 baseline]).

Urinary LTE_4 levels increased at two ($p=0.001$; 95%CI [2.087;4.948 baseline]), and four hours ($p=0.003$; 95%CI [1.931;4.579 baseline]) following aspirin challenge tests, as compared to baseline values, only in the group of patients with the positive aspirin challenge.

In the group of aspirin-intolerant patients, following celecoxib challenge urinary LTE_4 levels decreased at the time 0 ($p=0.02$; 95%CI [0.321;0.760 baseline]), and remained stable 2 (95%CI [0.320;0.762 baseline] and 4 hours later (95%CI [0.359;0.851 baseline]). In the group of aspirin-tolerant asthmatics there were no changes in urinary LTE_4 levels after celecoxib challenge, as compared to baseline values, fig 4.

There was a positive correlation between the urinary LTE_4 levels (at baseline, and following aspirin challenge), and the levels of blood eosinophil count only in aspirin-intolerant asthmatics (Spearman $r=0.78$; $p<0.001$)

Analysis of correlation between urinary LTE_4 and $\text{PGE}_2\text{-M}$ levels

Following aspirin challenge no correlation was found between urinary LTE_4 and $\text{PGE}_2\text{-M}$ levels in aspirin-intolerant patients.

There was a positive correlation between urinary LTE_4 levels, and the levels of $\text{PGE}_2\text{-M}$ following aspirin challenge (at 4 hours) only in aspirin-tolerant patients, (Spearman $r=0.63$; $p=0.006$).

Following placebo and celecoxib challenges no correlation was found in either study groups.

Analysis of correlation between urinary LTE₄ and tetranor-PGE-M levels

Following placebo, aspirin, and celecoxib challenges no correlation was found between urinary LTE₄ and tetranor-PGE-M levels in AIA ($p=0.99$, $p=0.27$, $p=0.58$; respectively) and ATA ($p=0.37$, $p=0.97$, $p=0.52$; respectively) patients.

DISCUSSION

In this study we measured both main metabolites in urine; PGE₂-M by enzyme immunoassay and tetranor-PGE-M by gas chromatography/mass spectrometry. The latter method discriminated between genuine PGE₂ metabolites and the PGE₁ end products depending on diet. It was reported that the mass spectrometry measurement of PGE₂ metabolites in urine is highly accurate and sensitive[31].

Our results demonstrate a similar urinary baseline level of PGE₂-M and tetranor-PGE-M in the three study groups: AIA, ATA, and healthy controls. In healthy subjects, as has been reported previously [32] level of both PGE₂ metabolites was significantly higher in men than in women. However, these differences were not observed by us in the asthmatic patients. Aspirin challenge performed in AIA patients did not change PGE₂-M and tetranor-PGE-M levels at any time during the observation period, as compared to baseline values. In contrast, in ATA patients, urinary levels of both PGE₂ metabolites became significantly depressed following two aspirin challenges of either 500 mg or 188 mg. There is a tentative explanation for a paradox finding of urinary PGE₂ metabolites not changing during the positive aspirin challenge in AIA. These metabolites decreased following comparable doses of aspirin in others, not aspirin-sensitive subjects. Constitutively expressed COX-1, by its main product PGE₂, controls activation of inflammatory cells. Upon inhibition with aspirin, it is the second source of PGE₂, i.e. COX-2, which takes part and contribute to urinary metabolites. This unmasking effect of aspirin was present only in AIA group, and as expected, celecoxib did not discriminate for aspirin-sensitivity, when urinary metabolites of PGE₂ were measured.

It has been postulated that COX-1 inhibition by aspirin, resulting in reduced PGE₂ production, provides mechanism that triggers attacks of asthma. [33] This hypothesis has been supported by the following data: 1/ inhalation of exogenous PGE₂ prevents bronchoconstriction provoked by aspirin and inhibits urinary excretion of cys-LTs, [16,17,33] 2/ epithelial cells from surgically removed nasal polyps of AIA patients produce less PGE₂ than the same cells from patients with ATA,[34] 3/ peripheral blood cells from AIA patients release less PGE₂ at baseline than healthy controls, [35] 4/ PGE₂ production by bronchial fibroblasts in aspirin-sensitive asthmatics is lower, as compared to patients with asthma who tolerate aspirin well, [36] 5/ lower expression of COX-2 in aspirin hypersensitive patients results in deficient production of PGE₂ in nasal polyp thus contributing to imbalance between eicosanoids, [37,38] 6/ inflammatory cells infiltrating the nasal mucosa of AIA patients are deficient in EP2 receptor, [39] 7/ of 370 SNPs from 63 candidate genes only a particular variant coding for EP2 was significantly associated with AIA. [40]

The evident discrepancy between the systemic biosynthesis of PGE₂, evaluated by its two main metabolites, and accumulating data for PGE₂ inhibition, as the triggering mechanism of AIA, could be explained by cellular mechanism of the disease. Hypersensitivity to aspirin and other drugs of its class is a phenomenon linked to a specific inflammation, [41] in which there is degranulation of mast cells in target organs, like bronchial wall, nasal and paranasal mucosa and skin in urticaria

patients. This has been repeatedly documented by increase of PGD_2 in blood,[41] [42] and urine, [43,44] histamine in nasal secretion, [45,46,47] bronchoalveolar lavage, [48] and tryptase in blood. [49] It is plausible that activated mast cells can augment biosynthesis of both PGE_2 and PGD_2 . However, only PGD_2 was reported in relation to the challenge. It is also likely that inflammatory mediators released from the activated mast cells, following a positive aspirin challenge could affect PGE_2 production through two mechanisms. First, they could upregulate PGE_2 biosynthesis in other cells through an intracellular calcium dependent phospholipase A_2 . This rise in systemic PGE_2 should depend on COX-2 activity and correlate with number of inflammatory cells. In fact, a selective COX-2 inhibitor diminished urinary levels of both PGE_2 metabolites to the same extent in AIA and aspirin-tolerant asthmatics. Second, they could modulate PGE_2 -related pathways. Indeed, in primary human airway epithelial cells IL-13 depresses PGE_2 production but at the same time up-regulates the activity of enzyme that metabolizes PGE_2 . [50] Thus, activity of the enzymes involved in PGE_2 degradation could be up-regulated by some of the numerous pro-inflammatory substances that are released during an aspirin-induced asthma attack. Additional evidence is based on up-regulation of cysLTs pathway in AIA. Mast cells along with eosinophils are the main systemic source of cysteinyl leukotrienes. The positive correlation between post-challenge increase of LTE_4 excretion and blood eosinophils count reflected mast cell – eosinophils functional unit, a hallmark of inflammation associated with cysLTs overproduction. [51] Some other factors could confound our results, though not undermine the postulates explaining the data.

In this study urinary LTE_4 levels behaved typically at baseline and following aspirin challenge as shown previously.[16,17] However, it was surprising to find a lack of correlation between urinary PGE_2 metabolites and LTE_4 levels in AIA patients. Moreover, the dose of aspirin had no effect on magnitude of the response of both PGE_2 metabolites and their duration in AIA patients.

In ATA group both placebo, ASA and celecoxib tended to diminish LTE_4 urinary excretion. In AIA patients similar trend was observed following placebo and celecoxib. These results point that aspirin and celecoxib in ATA and the latter in AIA group similarly to placebo do not affect the cys-LTs metabolism. The tendency of LTE_4 to decrease in time following placebo, aspirin and celecoxib administration in ATA and after placebo and celecoxib in AIA group may reflect the diurnal variation of cys-LTs production in the body. However, this observation would require further studies.

In only 1 out of 17 AIA patients studied, who developed shock following aspirin challenge, celecoxib triggered signs of bronchial obstruction, and a rise in urinary LTE_4 , while the remaining patients tolerated celecoxib very well. Thus, special care is necessary if celecoxib is to be given to patients with severe aspirin-intolerance. In the group of AIA patients, following celecoxib challenge urinary LTE_4 levels decreased, and remained stable at any time of the observation period. This result differs from earlier observations. [52] Contrary, to previous reports, [52] there were no changes in urinary LTE_4 levels after celecoxib administration, in the group of ATA patients.

In conclusion, this study describes the systemic production of PGE_2 in asthma and points to differences between aspirin-sensitive and aspirin-tolerant patients. The global production of PGE_2 remains unaffected by aspirin in aspirin hypersensitive patients as compared to asthmatics who tolerate aspirin well. A lack of depression of systemic PGE_2 biosynthesis following a challenge with aspirin in the hypersensitive

subjects, could be a consequence of mast cells activation with a secondary inflammatory response to the released mediators. However, this may not apply to local conditions in bronchi, where aspirin can suppress PGE₂ biosynthesis in AIA patients.

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Fig.1. Urinary PGE₂ metabolite (PGE₂-M – pg/mg creatinine) levels before and following placebo, aspirin, and celecoxib challenges in aspirin-induced asthma and aspirin-tolerant asthma patients (phase I).

- A. Placebo
- B. Aspirin
- C. Celecoxib

*** – $0 < p \leq 0.001$; ** – $0.001 < p \leq 0.01$; * – $0.01 < p \leq 0.05$

Fig.2. Urinary PGE₂ metabolites levels, before and following placebo, and the cumulative dose of 188 mg of aspirin in aspirin-tolerant asthmatics patients (phase II).

- A. PGE₂-M – pg/mg creatinine
- B. tetranor-PGE-M – ng/mg creatinine

*** – $0 < p \leq 0.001$; ** – $0.001 < p \leq 0.01$; * – $0.01 < p \leq 0.05$

Fig.3. Urinary PGE₂ metabolite (tetranor-PGE-M – ng/mg creatinine) levels before and following placebo, aspirin, and celecoxib challenges in aspirin-induced asthma and aspirin-tolerant asthma patients (phase I).

- A. Placebo
- B. Aspirin
- C. Celecoxib

*** – $0 < p \leq 0.001$; ** – $0.001 < p \leq 0.01$; * – $0.01 < p \leq 0.05$

Fig. 4. Urinary LTE₄ (pg/mg creatinine) levels before and following placebo, aspirin, and celecoxib challenges in aspirin-induced asthma and aspirin-tolerant asthma patients (phase I).

- A. Placebo
- B. Aspirin
- C. Celecoxib

*** – $0 < p \leq 0.001$; ** – $0.001 < p \leq 0.01$; * – $0.01 < p \leq 0.05$







