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Aspirin provocation increases 8-iso-PGE_2 in exhaled breath condensate of aspirin-hypersensitive asthmatics

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ABSTRACT

Background: Isoprostanes are bioactive compounds formed by non-enzymatic oxidation of polyunsaturated fatty acids, mostly arachidonic, and markers of free radical generation during inflammation. In aspirin exacerbated respiratory disease (AERD), asthmatic symptoms are precipitated by ingestion of non-steroid anti-inflammatory drugs capable for pharmacologic inhibition of cyclooxygenase-1 isoenzyme. We investigated whether aspirin-provoked bronchoconstriction is accompanied by changes of isoprostanes in exhaled breath condensate (EBC).
Methods: EBC was collected from 28 AERD subjects and 25 aspirin-tolerant asthmatics before and after inhalatory aspirin challenge. Concentrations of 8-iso-$PGF_{2\alpha}$, 8-iso-PGE_2, and prostaglandin E_2 were measured using gas chromatography/mass spectrometry. Leukotriene E_4 was measured by immunoassay in urine samples collected before and after the challenge.
Results: Before the challenge, exhaled 8-iso-$PGF_{2\alpha}$, 8-iso-PGE_2, and PGE_2 levels did not differ between the study groups. 8-iso-PGE_2 level increased in AERD group only ($p = 0.014$) as a result of the aspirin challenge. Urinary LTE_4 was elevated in AERD, both in baseline and post-challenge samples. Post-challenge airways 8-iso-PGE_2 correlated positively with urinary LTE_4 level ($p = 0.046$), whereas it correlated negatively with the provocative dose of aspirin ($p = 0.027$).
Conclusion: A significant increase of exhaled 8-iso-PGE_2 after inhalatory challenge with aspirin was selective and not present for the other isoprostane measured. This is a novel finding in AERD, suggesting that inhibition of cyclooxygenase may elicit 8-iso-PGE_2 production in a specific mechanism, contributing to bronchoconstriction and systemic overproduction of cysteinyl leukotrienes.

1. Introduction

An analysis of exhaled breath condensate (EBC) is a simple and noninvasive technique for monitoring airway inflammation. Lipid mediators derived from arachidonic acid, prostanoids and leukotrienes, can be measured in EBC and used as biomarkers in diseases such as asthma [1].

Isoprostanes are formed mainly during non-enzymatic peroxidation of arachidonic acid (AA), independently of enzymatic cyclooxygenase (COX) activity. As a result of free radical attack, various isomers of different prostaglandins, leukotrienes, and epoxyeicosatrienoic acids are generated [2]. Among them, there is 8-iso-$PGF_{2\alpha}$, also called 8-epi-$PGF_{2\alpha}$, which has been validated as a biomarker of oxidative stress [3]. In contrast with prostanoids synthesized in a trans-conformation by stereoselective enzymes, isoprostanes have cis-juxtaposition of the alkene residues at the cyclopentane ring. Because isoprostanes are stereoisomers of prostaglandins, they share the same chemical formulas. 8-iso-PGF_{2a} was measured in biological fluids including plasma, urine, cerebrospinal fluid, or bronchial lavage [4,5]. Studies on 8-iso-PGE_2, a cis-isomer of the most abundant prostaglandin E_2 (PGE_2), are not numerous because no convenient assay method is available. To date 8-iso-PGE_2 was detected in human biological samples and found to cause renal vasoconstriction and platelet activation [3]. Janssen et al. analyzed the effects of 8-iso-PGE_2 on the lungs [6]. 8-iso-PGE_2 constricted pulmonary vessels and bronchia. It was suggested that 8-iso-PGE_2 could be a ligand for thromboxane TP receptor, however, an affinity for PGE_2 receptors EP was also postulated [3]. Asthma is a clinical entity encompassing many phenotypes, with bronchial inflammation and airway hyperreactivity as common

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Table 1
Clinical characteristics of the study subjects.

	AERD ($n = 28$)	ATA ($n = 25$)	p Value
Age, years	46.14 ± 14.01	43.8 ± 11.48	N.S.
Gender, males/females (%)	12/16 (42.9)	13/12 (52)	N.S.
Duration of asthma, years	10 [5–15]	3 [1–13]	N.S.
Skin prick tests, positive/negative (%)	12/16 (42.9)	15/10 (60)	N.S.
Total blood eosinophil count, /mm^3	349 [149.25–574]	239 [128–479.75]	N.S.
Total serum IgE, IU/mL	55 [25.45–120.5]	59 [22.9–276]	N.S.
FEV_1, % of predicted value	99.1 [84.44–100.0]	98 [85.92–103]	N.S.
Asthma severity, NAEPP-EPR3	4 [3–4]	4 [3–4]	N.S.
Asthma control test score (ACT)	24 [22.8–25]	25 [23–25]	N.S.
ICS, yes/no (%)	25/3 (89.3)	21/4 (84)	N.S.
ICS dose, μg fluticasone equivalent	500 [362.5–1000]	500 [250–1000]	N.S.

Values are expressed as counts, arithmetic mean ± SD, and median [lower–upper quartile]. AERD–aspirin-exacerbated respiratory disease, ATA–aspirin-tolerant asthma, ICS–inhaled corticosteroids, N.S.–not significant.

features. Inflammation of the airways leads to increased levels of reactive oxygen species and formation of a variety of isoprostanes in asthmatics [7,8].

Aspirin-exacerbated respiratory disease (AERD) is a well-defined phenotype of asthma. It is characterized by a syndromic association of chronic rhinosinusitis with persistent asthma and aspirin hypersensitivity [9]. Prevalence of AERD in the general population is estimated 0.5%, whereas 10–15% of asthmatics have the syndrome. Bronchoconstriction in AERD develops following an ingestion of aspirin or other non-steroidal anti-inflammatory drugs. This asthmatic attack is triggered by a pharmacological inhibition of COX-1 isoenzyme, and is followed by a release of potent pro-inflammatory lipid mediators, including cysteinyl leukotrienes (cys-LTs) [10–12]. Urinary leukotriene E_4 ($uLTE_4$) is the most reliable biochemical biomarker of AERD and estimates the systemic overproduction of cys-LTs, also elevated in clinically stable asthmatics.

We previously reported on urinary metabolites of PGE_2: 13,14–dihydro-15keto-PGE_2 and tetranor-PGE-M in AERD, contrasted with aspirin-tolerant asthmatics (ATA), after oral aspirin administration [13]. Despite a similar systemic production of both PGE_2 metabolites at baseline, no decline in urinary PGE_2 metabolites was found following COX-1 inhibition exclusively in AERD subjects during the bronchoconstriction. However, a similar challenge decreased urinary excretion of PGE_2 metabolites in ATA subjects with negative outcome of the challenge. We realized that the analytical method for PGE_2 metabolites quantification was insensitive to cis/trans conformation of prostanoids.

Therefore, in the current study, we hypothesized that a free radical-mediated mechanism of prostanoid biosynthesis can contribute to the pathogenesis of aspirin hypersensitivity. We developed an assay based on gas-chromatography–mass-spectrometry enabling a separate quantification of PGE_2 and 8-iso-PGE_2 in biological fluids. In the current study, lysyl-acetylsalicylic acid (L-ASA) was administered by inhalatory challenge to provoke bronchoconstriction in hypersensitive AERD subjects. We analyzed EBC to avoid the background of abundant systemic biosynthesis of PGE_2. However, $uLTE_4$ excretion was measured concurrently to evidence the well known mechanism of bronchoconstriction in AERD. Subjects with AERD or ATA, whose clinical condition was stable, were enrolled to this study. To the best of our knowledge, this is the first study in which 8-iso-PGE_2 isoprostane is measured concurrently with PGE_2 in asthma.

2. Methods

2.1. Subjects

The study subjects comprised 28 AERD patients with a previously diagnosed hypersensitivity to aspirin using the oral challenge test, and 25 asthmatics who tolerated aspirin well. The diagnosis and evaluation of the disease control complied with Global Initiative for Asthma 2012 update. Severity of asthma was defined using ATS/ERS 2013 recommendations and was evaluated using National Asthma Education and Prevention Program's Expert Panel Report 3 guidelines [14]. Each subject was assigned the highest level of severity (intermittent, mild, moderate, or severe persistent) according to clinical features. Asthma Control Test was used to assess the disease control [15]. The clinical characteristics of the study patients are summarized in Table 1.

None of the study participants experienced an asthma exacerbation or a respiratory tract infection within 6 weeks preceding the study. During the study period all the patients had stable asthma and their baseline FEV_1 exceeded 70% the predicted value. The subjects were instructed to withhold any medication decreasing bronchial responsiveness prior to the challenge [16]. None of the patients was treated with systemic corticosteroids or leukotriene modifying drugs.

All study participants gave their written informed consent and the study protocol was approved by the Jagiellonian University Ethical Committee.

2.2. Study design

A single-blind, placebo-controlled bronchial challenge test with L-ASA (Kardegic, Sanofi Aventis) was carried out during one day in all participants of the study [16]. The test began with 7 inbreathes of saline. FEV_1 was monitored at 10 and 20 min after the placebo inhalation. The post-saline FEV_1 at 20 min was assumed as the baseline value. Incremental doses of L-ASA obtained by increasing the concentration of L-ASA or by changing number of breaths were inhaled every 30 min and equaled to 0.18, 0.36, 0.90, 2.34, 7.20, 16.2, 39.60, 115.20 mg. FEV_1 was measured at 10, 20, and 30 min after each dose. The challenge was interrupted if a bronchospastic reaction occurred (i.e., FEV_1 dropped by 20% or more), or the cumulative dose of L-ASA reached 181.98 mg. The cumulative dose of L-ASA causing 20% decrease in FEV_1 was the provocation dose (PD_{20}). FEV_1 and extrabronchial symptoms were recorded before and immediately after the challenge, then every 30 min for the next 6 h.

All the AERD subjects had EBC and urine samples collected before the challenge and at the time of the onset of bronchial symptoms. Additional samples of urine were collected 2 and 4 h after the challenge. In ATA group, EBC and urine samples were collected before the challenge and 30 min after the last dose of L-ASA, then additional urine samples were obtained as in AERD group.