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Systemic expression of inflammatory mediators in patients with chronic rhinosinusitis and nasal polyps with and without Aspirin Exacerbated Respiratory Disease

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ABSTRACT

Background: Systemic reactions are related to the pathogenesis of Aspirin Exacerbated Respiratory Disease (AERD). With this work we wanted to study the changes in the systemic levels of inflammatory mediators in both baseline and after oral aspirin challenge in patients with and without AERD.

Methods: Patients with nasal polyposis and asthma with AERD ($n = 20$) and without ($n = 18$) were orally challenged with aspirin in a single-blind placebo controlled study. Serum samples and urine were collected before and 6 h after placebo and aspirin oral challenges. Serum levels of inflammatory mediators were assayed by using the Luminex technology and ELISA. The concentrations of 9-alpha, 11-beta prostaglandin F_2, and leukotriene E_4 ($uLTE_4$) were measured in urine samples by ELISA. The expression of T-cell surface markers was analyzed in peripheral blood mononuclear cells isolated before and after the challenges.

Results: AERD patients showed significantly higher baseline levels of s-IL-5R-alpha, $uLTE_4$ and percentage of $CD4^+CD25^+CD127^{pos}$ and $CD4^+CD45RA^-CD45RO^+$ but decreased levels of TGF-β_1 and number of $CD4^+CD25^+CD127^{neg}$ cells. Aspirin challenge induced the release of $uLTE_4$, IL-6 and increased the number of $CD4^+CD45RA^-CD45RO^+$ memory T-cells only in AERD patients but failed to reduce the levels of sCD40L as observed in non-AERD subjects. Further, IL-8 and sIL-5R-alpha levels directly correlated with the PD_{20}ASA and the effects of aspirin on IL-6 and number of memory T-cells was more pronounced in subjects showing more strong reaction (bronchial and nasal).

Conclusions: AERD patients have a differential baseline inflammatory pattern that supports the role inflammation as underlying mechanism of the disease. Systemic response to oral aspirin challenge was related to an increase in serum IL-6 and the number of circulating memory T-cells in AERD patients.

1. Introduction

Aspirin and non-steroidal anti-inflammatory drugs (NSAIDs), one of the most commonly used group of drugs worldwide are involved in the pathogenesis of a condition defined as 'aspirin triad' or most recently Aspirin Exacerbated Respiratory Disease (AERD) [1]. This pathology is characterized by chronic eosinophilic rhinosinusitis with nasal polyposis, asthma and hypersensitivity

Abbreviations: 9α, 11β-PGF_2, 9 alpha, 11 beta prostaglandin F_2; AERD, Aspirin Exacerbated Respiratory Disease; CD127, Interleukin-7 receptor; Cys-LTs, cysteinyl leukotrienes; ENA78/CXCL5, C-X-C motif chemokine 5 or epithelial-derived neutrophil-activating peptide 78; Eotaxin/CCL11, chemokine (C-C motif) ligand 11 or Eotaxin; FEV_1, forced expiratory volume in one second; IgE, Immunoglobulin E; IP10/CXCL10, C-X-C motif chemokine 10 or Interferon gamma-induced protein 10 (IP10); ITAC/CXCCL11, C-X-C motif chemokine 11 or Interferon-inducible T-cell alpha chemoattractant; LXA_4, lipoxin A_4; 15-epi LXA_4, 15-epi-lipoxin A_4; MCP1/CCL2, chemokine (C-C motif) ligand 2 or monocyte chemotactic protein-1; MIP-1α/CCL3, chemokine (C-C motif) ligand 3 or macrophage inflammatory protein-1 alpha; MIP-1β/CCL4, chemokine (C-C motif) ligand 4 or macrophage inflammatory protein-1beta; PD_{20}ASA, provocative dose of aspirin that decreases the FEV_1 by 20%; S90k/MacBP, soluble mac-2 binding protein; sCD40L/sCD154, soluble CD40 ligand; s-IL-5Rα, soluble Interleukin-5 receptor alpha subunit; TGF-β_1, tumor growth factor beta 1; $uLTE_4$, urinary leukotriene E_4.

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reactions in both upper (nose and paranasal sinuses) and lower (lungs) airways after ingestion of aspirin and/or NSAIDs [1]. The pathomechanism of this disease has been attributed to the inhibition of cyclooxygenase-1 (COX-1) [1,2]. The inhibition of COX-1 probably results in the down-regulation of PGE_2 biosynthesis leading to increased production of cysteinyl leukotrienes (Cys-LTs) [3] that induce the precipitation of violent asthmatic attacks, which are usually accompanied by nasal blockage and ocular symptoms [4].

After intake, aspirin is primarily absorbed in the stomach and small intestine and later metabolized in the liver following the cytochrome P450 route [5]. Then the drug pass to the peripheral circulation where it encounters it cell targets: platelets, blood leukocytes and lymphocytes [5]. Systemic reactions after aspirin challenge, increased airway eosinophilia and influx of pro-inflammatory cells and mediators in patients with AERD were for years explained by imbalance of eicosanoids biosynthesis [2]. These studies demonstrated an increased production of pro-inflammatory Cys-LTs in bronchoalveolar lavage (BAL) fluid, urine, breath condensates and bronchial mucosa at both baseline and after aspirin challenge [6,7]. They also showed that AERD patients respond to aspirin challenge with a high production of 9 alpha, 11-beta prostaglandin F_2 (9α, 11β-PGF_2) [8] but with a down-regulation of lipoxin A_4 (LXA_4 and 15-epi-LXA_4) [9,10].

During the last decade, it has been observed that aspirin may influence the immune system. This has been supported by several studies showing the effect of the drug on the functionality of dendritic cells and T regulatory cells [11,12]. In the pathogenesis of AERD only a few research groups have addressed this subject. Lee et al. [13] showed that aspirin challenge lead to a significant increase of plasma concentrations of complement component C3a only in patients with AERD. Further, Makowska and colleagues reported that baseline serum levels of Eotaxin-2 were up-regulated in AERD patients and bronchial aspirin challenge resulted in a marked increase of the number of leukocyte ($CD34^+$) and eosinophil ($CD34^+CD125^+$) progenitor cells in AERD subjects with systemic reactions [14]. Moreover, nasal administration of lysine aspirin may significantly increase the levels of CCL5 and ECP in nasal secretions from AERD patients but not non-AERD subjects [15].

It has been shown that the manifestation of AERD may be linked to underlying inflammatory processes that might predispose for further hyper-responsiveness reactions to aspirin or NSAIDs. In this study, we evaluated the systemic expression of inflammatory mediators at baseline and after oral aspirin challenge and the possible relationship with the clinical parameters observed in the study patients.

2. Materials and methods

2.1. Study subjects and clinical diagnosis

For this study, patients with chronic rhinosinusitis with nasal polyposis (CRSwNP), asthma with AERD ($n = 20$) and without AERD (non-AERD, $n = 18$) were recruited at the Jagiellonian University Medical College in Cracow, Poland. The clinical data of the patients is summarized in Table 1. Asthma was diagnosed according to the Global Initiative for Asthma (GINA) guidelines [16]. Chronic rhinosinusitis with nasal polyposis was evidenced based on medical history, endoscopic and/or computed tomography (CT) scan findings as described in the EPOS guidelines [17]. In order to confirm or exclude aspirin hypersensitivity, a single blind, two-days, placebo-controlled, aspirin oral challenge was conducted in all patients as described in EAACI/GA^2LEN guidelines [18].

Doses of oral, inhaled (bronchial) and nasal corticosteroids were as low as possible (less than 10 mg of oral prednisolone equivalent in case of oral corticosteroids) and kept stable throughout the duration of the challenge. Other drugs were withheld before challenge according to the EAACI/GA^2LEN guidelines [18] (Table 2). The study was initiated after obtaining approval by the Bioethical Committee of the Jagiellonian University Medical College in Cracow, Poland and signed informed consent by all patients.

2.2. Protocol for oral aspirin challenge

The first day of the protocol consisted in challenge with a placebo (*Saccharin lactate*) that was administrated four times every 1.5 h. The placebo challenge aimed to exclude false-positive reactions and to assess upper and lower airway stability. The patients were excluded from further aspirin challenges when the forced expiratory volume in one-second (FEV_1) fall exceeded 10%, compared to baseline FEV_1. On the second day, patients received every 1.5 h four increasing aspirin doses (27, 44, 117 and 312 mg) up to a cumulative dose of 500 mg. Clinical symptoms, such as dyspnea, rhinorrhea, nasal congestion, sneezing, ocular injection or skin flushing/urticaria, were inspected every 30 min. Serial spirometry

Table 1
Clinical characteristics of the study patients. Data are expressed in median and interquartile range (IQR).

	AERD patients ($n = 20$)	Non-AERD patients ($n = 18$)	*P*-value
Age (years)	46.0 (35.0–54.0)	44.0 (32.0–51.0)	$P > 0.05$
Gender (ratio female/male)	15/5	13/5	$P > 0.05$
FEV_1 in% predicted (standard deviation)	94.2 (SD: 15.8)	88.3 (SD: 9.2)	$P > 0.05$
Rhinosinusitis duration (years)	13.0 (2.0–25.0)	11.0 (2.0–40.0)	$P > 0.05$
PD_{20} ASA (mg)	330.0 (145.0–475.0)	Not applicable	
Asthma duration (years)	9.0 (1.0–21.0)	5.0 (2.0–10.0)	***P = 0.0235***
Duration of nasal polyps (years)	8.0 (1.0–19.0)	1.5 (1.0–25.0)	$P > 0.05$
Duration of AERD (years)	8.0 (5.5–14.0)	Not applicable	
Number of polypectomies	3 (0–10)	1 (0–5)	***P = 0.06***
Number of allergic patients	11/20	10/18	$P > 0.05$
Inhaled corticosteroids (mcg)[a]	800.0 (800.0–1200.0)	800.0 (300.0–800.0)	N.S.
Nasal corticosteroids (mcg)[b]	100.0 (100.0–200.0)	100.0 (100.0–150.2)	N.S.
Presence of *S. aureus* in nasal swabs (% of positive patients)	35.0	21.4	N.S.
Serum total IgE (IU/ml)	68.0 (33.0–144.0)	107.0 (71.0–180.7)	N.S.
Blood eosinophils count (cells/mm^3)	509.0 (384.0–913.0)	340.0 (195.7–438.0)	***P = 0.034***
Peak inspiratory nasal flow (mL/min)	122.7 (65.0–180.0)	186.7 (140.0–220.0)	***P = 0.004***

AERD: patients with Aspirin Exacerbated Respiratory Disease, non-AERD: patients without aspirin exacerbated disease; FEV_1: forced expiratory volume in 1 s; PD_{20}ASA: dose of aspirin provoking a fall in FEV_1 of 20% or more; SD: Standard deviation of the mean; mcg: micrograms; mg: milligrams; *P*-value: represent the significance after Mann–Whitney test.
The values in bold italics represent $p < 0.05$.
[a] Re-calculated as equivalent of Budesonide.
[b] Re-calculated as equivalent of Prednisolone.