



Short communication

Aspirin desensitization in aspirin-exacerbated respiratory disease: New insights into the molecular mechanisms

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ABSTRACT

Background: Aspirin desensitization (AD) has been the only available modifying treatment in aspirin-exacerbated respiratory disease (AERD). The mechanisms of AD are nonetheless poorly understood. Though very effective, AD is limited by its risks and side-effects.

Objective: Moving forward to the targeted biologicals era, the aim of this study was to characterize the airway inflammatory response to long-term AD, including TSLP dynamics, in order to assess potential new targets in AERD.

Patients and methods: Adult patients with aspirin challenge-confirmed AERD underwent an oral AD followed by daily ingestion of aspirin for at least 6 months. Clinical data and inflammatory biomarkers were measured and compared, before and after AD. Induced sputum analyses were performed at baseline, one and six months after AD (differential cell count and levels of sputum supernatant leukotriene C4, prostaglandin D2 and E2, and TSLP). **Results:** AD was followed by significant clinical improvement, as quantified by all monitored parameters. The good clinical outcomes of AD in our study are supported by overall changes observed in the arachidonic acid metabolites (decreased PGD2 over a constant LTC4/PGE2). TSLP increased (mean baseline 0.1 ± 0.03 ; 1 month 3.68 ± 7 ; 6 months 212.2 ± 44 pg/ml; $p < 0.01$).

Conclusions: Our findings suggest that new biologicals blocking TSLP might have a clinical benefit in AERD, by cutting down the TSLP-induced PGD2 generation.

1. Introduction

The pathogenesis of aspirin-exacerbated respiratory disease (AERD) is based on complex dysregulations within the arachidonic acid metabolic pathways, partly genetically determined [1,2]. The end-result is an imbalance between overproduction of proinflammatory and bronchoconstrictor eicosanoids, especially leukotriene C4 (LTC4) and prostaglandin D2 (PGD2), and a defective generation of anti-inflammatory regulators, especially prostaglandin E2 and lipoxins. The aspirin-sensitive asthma endotype is thus characterized by extensive eosinophilic, T2-high airway inflammation and increased mast-cell degranulation [1,2].

Within the last 30 years, aspirin desensitization (AD) has been the

only available modifying treatment in otherwise difficult to control patients with severe, steroid-dependent asthma, and/or recurrent nasal polyposis. Numerous studies have demonstrated the beneficial clinical effects of AD, most of them open trials without a placebo arm [3], and recently a double-blind placebo-controlled trial [4]. The mechanisms of AD are poorly understood. Previous small studies failed to unequivocally prove changes in the eicosanoids metabolism, but others demonstrated that long-term AD involved suppression of IL-4, as a consequence of direct inhibition of tyrosine kinases and the signal transducer and activator of transcription 6 signalling pathway (STAT 6), while Th1 markers increased [5].

Recently, the epithelial-derived cytokine thymic stromal lymphopoietin (TSLP) has been identified as a potent inducer of T2-type

Abbreviations: AD, aspirin desensitization; AERD, aspirin-exacerbated respiratory disease; FeNO, Fractional exhaled nitric oxide; LTC4, leukotriene C4; ppb, parts per billion; PGD, PGE2, prostaglandin D and E2 respectively; T2, type 2 immune response; TSLP, Thymic stromal lymphopoietin

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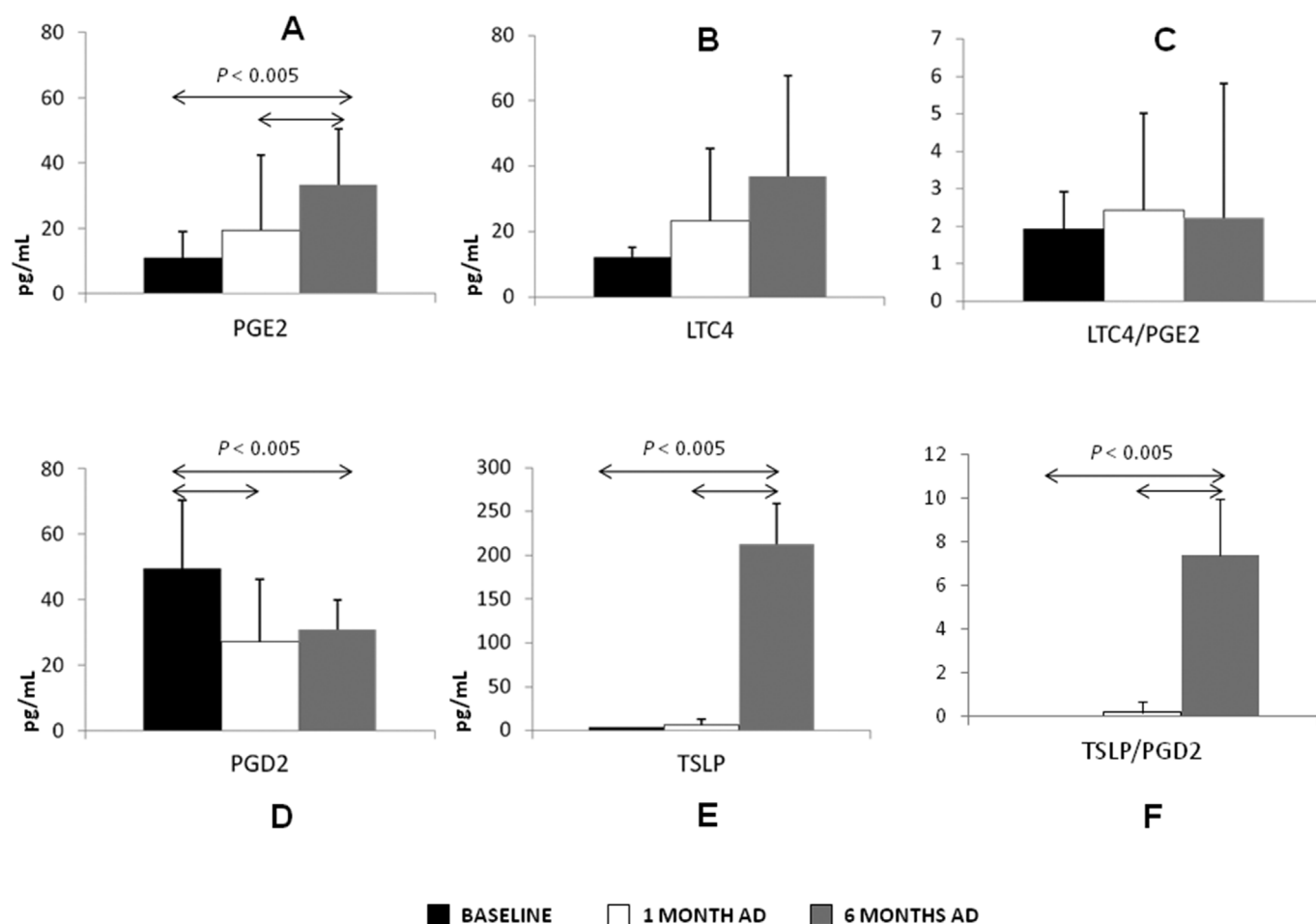


Fig. 1. Dynamics of induced sputum biomarkers.

Sputum eicosanoids and TSLP levels baseline and after AD (pg/mL): PGE₂ (A); LTC₄ (B); LTC₄/PGE₂ ratio (C); PGD₂ (D), and TSLP (E). AD: aspirin desensitization.

Data expressed as geometric mean $\pm$ SD (pg/mL). Statistical analysis was performed by Mann-Whitney *U* test.

responses. TSLP genetic variants have been linked to atopic dermatitis, asthma, and eosinophilic esophagitis [6]. In AERD, mast cell-derived PGD₂ is a major effector of T2 immune responses driven by TSLP. The dysregulation of this innate system contributes significantly to the pathophysiology of AERD, as reported recently [7].

Though very effective, AD is limited in clinical practice by its risks and side-effects. Moving forward to the targeted biologicals era, the aim of this study was to characterize the airway inflammatory response to long-term AD, including TSLP dynamics, in order to assess potential new targets in AERD.

2. Patients and methods

This was a prospective, interventional, open study, approved by the Ethics Committee of Hospital La Paz (Madrid, Spain). All patients provided written informed consent to their participation in the study. Adult patients with aspirin challenge-confirmed AERD [1], who had AD indications and no contraindications, underwent an oral AD over a 3-day period, followed by daily ingestion of aspirin starting with 650 mg bid during one month, then tapering the dose in each case [3]. The AD was initiated when each patient's asthma was controlled [3], and at least after one month from the last asthma exacerbation/respiratory tract infection, in order not to interfere with the inflammatory biomarkers measured. Demographic and clinical data were collected: age, sex, body mass index (BMI), smoking status, AERD-related data (age of onset, asthma severity, number of sinus surgeries and episodes of

bacterial sinusitis/year). The following items were further assessed at baseline (before AD) and on a monthly basis after AD: nasal symptoms, asthma control and severity according to current guidelines, controller asthma treatment, Asthma control test (ACT), quality of life (RSDI, AQLQ), spirometric parameters, FeNO. Induced sputum analyses were performed at baseline, one and six months after AD (differential cell count and levels of sputum supernatant leukotriene C₄, prostaglandin D and E₂, and TSLP). Data were analysed with SPSS v11.0 software (SPSS, Inc., Chicago, IL).

We chose to measure inflammatory biomarkers in sputum as it is far less invasive than bronchial biopsy or bronchoalveolar fluid, but still more organ-targeted than systemic biomarkers. Sputum induction was performed using inhalation of hypertonic saline 4.5% for a maximum of 20 min, via ultrasonic nebulizer (Omron NE-U17); the sputum samples were collected, processed and examined by means of cytospin and visual count, following European Respiratory Society recommendations [8], within 2 h after induction. Prostaglandin D₂, E₂ and cysteinyl-leukotriene C₄ were measured by specific ELISA Kits (Cayman Chemical Company, Ann Arbor, MI), as well as TSLP (R&D Systems, Minneapolis, MN). The intra-assay coefficient of variation was between 4% and 8%, and the inter-assay coefficient was between 4% and 10%, with a specificity of 100%. The lower detection limit of these assays was 8.26 pg/mL (PGE₂), 17.1 pg/mL (LTC₄), 19.5 pg/mL (PGD₂) and 3.46 pg/mL (TSLP) respectively.

3. Results

Thirteen out of fourteen patients underwent successful AD, but for different reasons only 11 of them reached 6 months of aspirin treatment. From these 11 patients, one of them needed addition of omalizumab in order to control the aspirin-induced associated urticaria, otherwise uncontrollable while taking aspirin daily [9]; so she was excluded from the biomarkers analysis. Thus, we eventually analysed 10 patients (6 females, 4 males, mean age 44 [21–65] years) (see also Table 1 for individual data). 5 had moderate asthma but severe recurrent nasal polyps (number of endoscopic surgeries previous to AD: 3[1–7]/patient), and the other 5 patients had severe steroid-dependent asthma and chronic rhinosinusitis with recalcitrant nasal polyps. All clinical outcomes improved 1 and 6 months after AD: ACT (16, 23, 24 points; $p < 0.05$); AQLQ (4, 6.3, 6.8 points; $p < 0.05$); VAS (58, 45, 38 mm; $p < 0.05$); FEV1 (79, 85, 86% of predicted; ns). Also, mean FeNO (53, 38, 32 ppb; $p < 0.05$) and sputum eosinophilia decreased as compared to baseline (ns, data not shown), in spite of significant reduction in the required dose of inhaled corticosteroids (initial median dose of fluticasone: 846 µg/day; 1 month after AD: 700 µg/day; 6 months: 400 µg/day, $p < 0.005$).

Sputum PGD2 decreased 1 and 6 months after AD compared to baseline ($p < 0.05$), and PGE2 increased 1 and 6 months after AD ($p < 0.05$), but LTC4 also increased slightly (ns), thus maintaining a constant LTC4/PGE2 before and after AD (see Fig. 1 A, B, C, D, Table 2). TSLP levels increased 1 and 6 months after AD in all patients (mean baseline 0.1 ± 0.03 ; 1 month 3.68 ± 7 ; 6 months 212.2 ± 44 pg/ml; $p < 0.01$ compared to baseline and to 1 month) (Fig. 1E, Table 2). The TSLP/PGD2 ratio significantly increased ($p < 0.001$): 0.003 ± 0.002 ; 0.202 ± 0.5 ; 7.38 ± 2.66 at baseline, 1 and 6 months after AD respectively (see Fig. 1F).

4. Discussion

In our study, the good clinical outcomes are supported by the overall changes in the arachidonic acid metabolites (decrease of PGD2 on a constant LTC4/PGE2 ratio). Surprisingly, TSLP increased 6 months after AD, in spite of the clinical and lung function improvement. So, in light of recent findings, we hypothesize that TSLP can act not only as an effector molecule, but also as a regulator [6,7]. As recently demonstrated by Buchheit et al. [7], TSLP controls prostaglandin D2 generation in patients with AERD; so the increase in TSLP in our study might be due to a simple negative feed-back effect, in response to the decrease of PGD2, as supported by the TSLP/PGD2 ratio; all within the complexly dysregulated innate response of these patients.

Our findings need further validation in larger studies and with a control group of tolerant asthmatics, in order to exclude a direct effect of aspirin *per se*. Nevertheless, clinical trials with TSLP downstream blockade are currently underway in allergic asthma [6], and our findings suggest that blocking TSLP might also have a clinical benefit in AERD, by cutting down the TSLP-induced PGD2 generation. As PGD2 is

dramatically increased in the most severe AERD subphenotypes [10], anti-TSLP might result particularly effective in these patients, as well as PGD2 receptor antagonists, also in phase III clinical trials. In the meantime, particularly in countries where new biologicals are hardly available due to high costs, aspirin desensitization should still be regarded as a valid option for candidate patients.

Conflicts of interest

The authors have nothing to declare.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at <https://doi.org/10.1016/j.rmed.2018.08.009>.

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Table 1. Baseline characteristics of the patients.

Patient	Demographics		Asthma characteristics						Rhinosinusitis/nasal polyps	
	Age	Sex	Severity	ICS dosis *	Other controllers	FEV1	ACT	AQLQ	Nº Synus surgeries	VAS nasal symptoms
1	62	male	moderate	750	Montelukast 10 mg, Salmeterol	72	19	4.5	3	68
2	29	female	severe	1000	Montelukast 10 mg, Salmeterol	92	8	3.5	1	44
3	58	male	severe	1000	Montelukast 10 mg, Salmeterol	68	15	4	7	72
4	27	female	severe	1000	Montelukast 10 mg, Salmeterol	98	11	1.8	2	42
5	43	female	moderate	204	Montelukast 10 mg, Formoterol	91	19	4	2	45
6	39	female	severe	1000	Montelukast 10 mg, Salmeterol	72	14	4.2	2	59
7	21	male	moderate	750	Montelukast 10 mg, Salmeterol	81	21	6	2	55
8	36	female	moderate	500	Montelukast 10 mg, Salmeterol	79	19	5.5	3	62
9	65	male	severe	1500	Montelukast 10 mg, Salmeterol	69	14	3	4	59
10	60	female	moderate	750	Montelukast 10 mg, Salmeterol	68	20	3.5	4	74

ACT: asthma control test, points; AQLQ: asthma quality of life questionnaire, points; *ICS dosis: inhaled corticosteroids expressed as µg fluticasone or equivalent; VAS: visual analogic scale for nasal symptoms, mm.

Table 2. Dynamics of the biomarkers before and after aspirin desensitization.

Biomarker		LTC4			PGE2			PGD2			LTC4/PGE2			TSLP		
Patient		Baseline	1 month	6 months	Baseline	1 month	6 months	Baseline	1 month	6 months	Baseline	1 month	6 months	Baseline	1 month	6 months
	1	7,89	12,724	9,365	4,4181	5,689	60,312	45,302	40,841	39,0174	1,1	1,04	35,47	0,1	0,1	195,707
	2	15,03	19,874	73,694	6,744	2,723	22,692	53,71	31,927	16,917	1,68	1,88	10,069	0,1	0,1	212,439
	3	97,54	98	100,517	4,785	64,877	8,418	76,465	61,803	27,982	1,275	1,593	21,94	0,1	2,23	218,016
	4	12,256	11,097	8,7589	24,836	4,546	23,854	94,757	11,783	44,868	0,493	0,26	91,01	0,1	0,1	220,551
	5	13,24	66,807	32,235	4,217	29,788	50,481	16,671	19,1748	28,439	0,86	0,67	33,06	0,1	0,1	227,142
	6	9,249	1,268	5,787	9,532	0,819	23,498	30,367	4,564	39,0174	6,65	0,11	33,06	0,2	0,1	184,045
	7	13,063	23,63	38,685	3,426	5,568	19,688	64,667	41,355	28,439	1,53	1,45	18,58	0,1	17,234	292,549
	8	11,586	14,458	28,689	5,365	21,579	39,859	56,022	14,579	19,903	3,84	0,73	5,18	0,1	0,1	117,625
	9	19,664	6,394	5,478	23,854	19,88	27,456	50,5	11,698	8,456	4,31	1,38	1,96	0,1	0,1	211,048
	10	7,212	22,565	33,503	16,04	47,023	51,488	33,0273	12,393	33,596	2,66	0,36	12,63	0,1	16,727	243,367

Data expressed as geometric mean (pg/ml).