

Original article

Lipoxin A₄ generation is decreased in aspirin-sensitive patients in lysine-aspirin nasal challenge *in vivo* model

Background: Lipoxins represent a group of lipoxygenase derived eicosanoids which, in contrast to leukotrienes, are potent anti-inflammatory mediators. The aim of our study was to determine lipoxin A₄ (LXA₄) and leukotriene C₄ (LTC₄) levels in nasal lavages after intranasal challenge with aspirin in aspirin intolerant (AIA) in comparison to aspirin tolerant (ATA) asthmatics and after allergen challenge in patients suffering from allergic rhinitis.

Methods: Twelve AIA, 8 ATA and 20 allergic patients were challenged with placebo, 16 mg of lysine-aspirin (Lys-ASA) or allergen (grasses). Nasal lavages were collected and eicosanoids' levels were determined using ELISA.

Results: Clinically positive Lys-ASA challenge in AIA resulted in influx of leukocytes (eosinophils and basophils) to nasal secretions and increase of LTC₄ to 106.82 pg/ml ($P < 0.05$ vs baseline (26.58 pg/ml)) on first hour after the challenge. We did not observe any differences in LTC₄ level before and after ASA challenge in ATA. In AIA group the mean level of LXA₄ was 43 ± 21.5 pg/ml after placebo and decreased in 2 h after Lys-ASA challenge (29 ± 17 pg/ml, $P = 0.015$). We found an increase in LXA₄ in ATA after ASA provocation as compared to placebo (33 ± 16 pg/ml vs 52 ± 31 pg/ml, $P = 0.046$). In atopic patients baseline level of LXA₄ was 33.49 ± 16.95 pg/ml with no difference after the clinically positive allergen challenge (36.22 ± 13.26 pg/ml, $P = 0.23$).

Conclusions: Results of our study confirm that AIA have diminished LXs' biosynthesis capacities *in vivo* after ASA challenge. Taking into consideration anti-inflammatory properties of lipoxins this phenomenon may be partially responsible for the development of chronic inflammation in AIA patients.

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Lipoxins (LXs) are lipoxygenase (LO) products of arachidonic acid metabolism which, in contrast to other eicosanoids including leukotrienes and prostaglandins, are recognized to have potent anti-inflammatory and pro-resolution properties. Lipoxins inhibit chemotaxis, transmigration across endothelial and epithelial monolayers, diapedesis and superoxide anion generation by polymorphonuclear leukocytes (PMN) (1). LXA₄ decreases leukotriene B₄ (LTB₄) release by human neutrophils and tumour necrosis factor alpha-induced interleukin (IL)-1 β production by PMN (2). Produced locally at the site of inflammation by transcellular interactions between leukocytes and resident cells in human tissues (epithelial cells, platelets and fibroblasts) during inflammation, LXs serve as a stop signal for inflammatory response and thus limit damage to the host. Several animal models demonstrated that LXs play an important role in orchestrating anti-inflammatory processes including peritonitis, asthma and dermal inflammation (3). First human studies showed that LXs

levels are significantly diminished in airway inflammation, cystic fibrosis and glomerulonephritis. Recently, Planaguma et al. (4) proved that patients with severe asthma have significantly less LXA₄ in bronchoalveolar lavage fluid than subject with nonsevere asthma. Moreover levels of cysteinyl leukotrienes were increased which may lead to an imbalance in eicosanoid mediators towards those that promote the airway inflammation. LXA₄ receptor gene and protein expression were also significantly decreased in peripheral blood granulocytes from severe asthmatics.

Aspirin (ASA) intolerance is a clinical syndrome associated with usually severe and chronic inflammation both in upper and lower airways resulting in chronic rhinitis, sinusitis, recurrent polypoidosis and asthma (5). Overproduction of cysteinyl leukotrienes (LTC₄, LTD₄ and LTE₄), which are potent proinflammatory mediators and bronchoconstrictors, is typical for pathophysiology of aspirin-intolerant asthma. Thus the role of potentially protective lipoxins in this disease should not be underestimated.

Sanak et al. (6) evaluated the capacity of the whole blood samples from aspirin-intolerant asthmatics (AIA), aspirin-tolerant asthmatics (ATA) and healthy subjects to generate LXA₄, 15-epi-LXA₄ and LTC₄. Formation of LTC₄ was similar in both AIA and ATA whereas significantly higher levels of LXA₄ were produced in ATA than AIA samples. This *in vitro* model suggests that diminished capacity for LXs synthesis by AIA may contribute to their more severe clinical phenotype, however available *in vivo* data are inconclusive. Up to date only one study evaluated LXA₄ generation *in vivo* in aspirin challenge model. Levy et al. (7) found that oral ASA challenge provoked statistically significant increase of LXA₄ levels in nasal lavage fluids of AIA patients. As this is in clear contrast to the Sanak's (6) paper we designed a protocol further investigating this problem.

The aim of the present study was to evaluate the capacity of aspirin-intolerant patients in comparison to aspirin-tolerant subjects to synthesize LXA₄ and LTC₄ in lysine aspirin (Lys-ASA) nasal challenge *in vivo* model.

Methods

Study subjects

Twelve aspirin sensitive (AIA), eight aspirin tolerant asthmatics (ATA) and 20 patients suffering from seasonal allergic rhinitis allergic to grasses were included into the study (Table 1). Patients' medical histories were studied, demographic and clinical data were collected. The diagnosis of aspirin intolerance has been confirmed with an oral aspirin challenge performed during the 12 months directly preceding the study according to accepted protocols (8). All ATA patients reported use of nonsteroidal anti-inflammatory drugs (NSAIDs) without any adverse effects. Patients with aspirin hypersensitivity had statistically significant lower baseline FEV₁ (forced exhaled volume in first second) values (3.59 vs 3.96, respectively, $P = 0.048$) and higher number of polypectomies (2.55 vs 0.72, respectively, $P = 0.033$) in comparison to ATA group. All patients were nonsmokers, in stable clinical condition. The doses of

asthma medications including inhaled steroids and rescue drugs were not changed during the study. None of the subjects had received any systemic steroids. Topical steroids and oral antihistamines were withdrawn for at least 2 weeks, antileukotrien drugs – 1 week, nasal α -agonists – 48 h before the challenge.

Nasal challenge

Intranasal challenges were performed with placebo (saline solution) or single dose (16 mg) of Lys-ASA (Aspisol®; Bayer, Leverkusen, Germany, Germany) aerosolized to both nostrils on a consecutive day. Aspirin dose was selected basing on previous studies (9, 10). Patients suffering from allergic rhinitis were challenged with allergen (grasses 5000 SBE/ml) (Allergopharma, Reinbeck, Germany) or placebo. Nasal lavages were collected before and then 1, 2, 4 and 24 h after challenges as described by Greiff et al. (11). A centrifugation (10 min at 0.08 g, 4°C) of saline washings separated cell pellet and supernatant. Supernatant has been immediately frozen (–80°C) for further analyses. The obtained sediment was washed in a sterile phosphate buffered saline (PBS, Sigma) and then suspended in 1.0 ml of RPMI 1640 (Sigma). After staining the cells (Hansel's method) the total number of eosinophils and basophils (metachromatic cells) was counted with the use of Fuchs-Rosenthal chamber allowing determination of the number of cells in 1.0 ml of recovered fluid. Differential cell counts were performed on slides stained by May–Grunwald–Giemsa method. A minimum of 400 cells was counted per smear to enable differential cell count to be made for each specimen. Cells were classified according to their morphology as neutrophils, eosinophils, basophils, mononuclears and epithelial cells. The epithelial cells were disregarded.

Clinical symptoms (rhinorrhea, itching nose and eyes, sneezing, blockage, postnasal drip) were described by patients before and after the challenge with visual analogue scale (VAS) (12) and then transferred to numerical values (0 – minimum, 100 – maximum points for one symptom, resulting in 600 points in total) for further analyses. All other symptoms including but not restricted to cough, dyspnoe, edema, adverse events and lung function tests were also recorded.

Measurement of mediators

Leukotriene C₄ levels were determined in nasal lavage fluid at all time points using ELISA method (Cayman Chemical, Ann Arbor, MI, USA, kit's detection limit 10 pg/ml). Lipoxin A₄ levels were determined before and 2 h after the challenge using ELISA method (Neogen Corp., Lansing, MI, USA, kit's detection limit 20 pg/ml).

Statistical analysis

Collected data were analyzed using spss 17.0 (Chicago, IL, USA) with chi-square test, Fisher's test, Wilcoxon's test (within groups) and Mann–Whitney *U*-test for comparison between the groups. ROC curve has been drawn. *P*-values of less than 0.05 were considered as significant. The study protocol was approved by the local ethic board of the Medical University of Lodz and informed consent was obtained from all subjects.

Results

Intranasal Lys-ASA challenge in ASA sensitive patients induced rhinorrhea, sneezing, nasal congestion, itching

Table 1. Basic characteristic of the study group

	AIA	ATA	<i>P</i> (AIA vs ATA)	Allergic
<i>n</i>	12	8	–	20
Age (years ± SD)	43.98 ± 11.34	37.45 ± 12.03	NS*	35.66 ± 10.78
Sex (F : M)	7 : 5	5 : 3	NS†	11 : 9
Atopy§	7	4	NS‡	20
FEV ₁ (l/min)	3.59	3.96	$P = 0.048^*$	–
FEV ₁ (% predicted)	74%	97%	–	–
Polypectomies (mean, median, min–max)	2.55 (0; 0–8)	0.72 (0; 0–2)	$P = 0.033^*$	0

NS – $P > 0.05$.

*Mann–Whitney *U*-test.

†Chi-square test.

‡Fisher's test.

§Atopy defined as at least one positive result (≥ 3 mm) in skin prick tests with a basic allergen set.

and sometimes cough. Mean value of symptoms increased in AIA group from 53.75 at baseline to 178, 161.75, 112.75 and 102 (at 1, 2, 4 and 24 h respectively) ($P < 0.05$, Wilcoxon's test). Neither placebo challenge in AIA group nor Lys-ASA and placebo challenges in ATA group induced significant increase in symptoms at any time point ($P > 0.05$, Wilcoxon's test). One patient from AIA group complained about dyspnoea with significant drop in FEV₁ ($\Delta 18.6\%$) after ASA challenge. This resulted in treatment with nebulized short acting β agonists (salbutamol) and oral steroids (a single dose of 32 mg of methylprednisolone). Other adverse events in AIA group after Lys-ASA challenge were mild and did not require any medications. Patients reported cough (three subjects), throat itching (two subjects), headache (two subjects) and face redness (one subject). No adverse events were reported by ATA subjects and allergic patients.

Symptoms of clinically positive nasal challenge were accompanied by influx of leukocytes to nasal secretions. The cells' influx started on the first hour after provocation, reached maximum in second hour and prolonged in some cases to 24 hours. Percentage of eosinophils after Lys-ASA challenge increased in AIA patients from 3.5% (mean basal value) to 15.25% in first hour, 17.05% in second, 11.4% in fourth and 8.45% in 24th hour. Maximal spontaneous influx in placebo day was observed in second hour and reached 6.9% (Fig. 1). Percentage of basophils after Lys-ASA challenge increased in AIA patients from 1.5% (mean basal value) to 3.3% in 1st hour, 5.1% in second, 4.95% in fourth and 2.5% in 24th hour. Maximal spontaneous influx in placebo day was observed in fourth hour and reached 2.15%. The influx of both eosinophils and basophils was significantly higher in AIA patients as compared Lys-ASA to placebo challenge ($P < 0.05$) and in AIA as compared to ATA group ($P < 0.05$).

Levels of leukotrienes and lipoxins

In AIA group after Lys-ASA challenge the mean level of LTC₄ increased from 26.58 pg/ml at baseline to 106.82,

52.81, 49.46 and 57.16 pg/ml, in first, second, fourth and 24th hour time points ($P = 0.001$, 0.0041, 0.004 and 0.017, baseline vs consecutive time points respectively, Wilcoxon's test). In placebo day significant increase was found at fourth hour time point only (29.79 vs 53.5 pg/ml, baseline vs fourth hour respectively, $P = 0.008$, Wilcoxon's test). Neither placebo nor Lys-ASA challenge induced any statistically significant changes in LTC₄ levels in ATA group (Fig. 2). When both study groups were compared statistically significant higher levels of LTC₄ were found in AIA in comparison to ATA at baseline ($P = 0.045$, Mann-Whitney *U*-test) and in first hour time point ($P = 0.016$, Mann-Whitney *U*-test).

In AIA group the mean level ($\pm$ SD) of LXA₄ was 43 ± 21.5 pg/ml after placebo and decreased after Lys-ASA challenge (29 ± 17 pg/ml, $P = 0.015$, Wilcoxon's test). We found statistically significant increase in LXA₄ level in ATA group after ASA provocation as compared to placebo (33 ± 16 and 52 ± 31 pg/ml after placebo and ASA respectively, $P = 0.046$, Wilcoxon's test) (Fig. 3A). The comparison between AIA and ATA group revealed statistically significant difference in LXA₄ levels after Lys-ASA challenge ($P = 0.044$) but not after placebo ($P = 0.266$). Moreover, the relationship between LX and LT production, expressed as the ratio of LXA₄ to

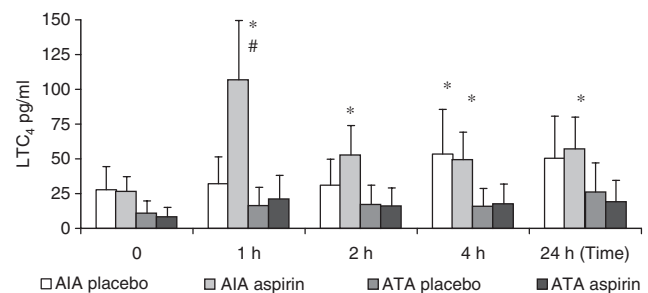


Figure 2. LTC₄ levels (pg/ml) in nasal lavage fluids after Lys-ASA challenge (* $P < 0.05$ baseline vs consecutive time points, # $P < 0.05$ placebo vs Lys-ASA challenge).

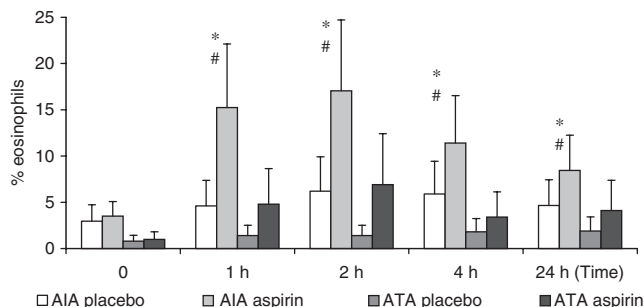


Figure 1. Eosinophils influx (% $\pm$ SD) into nasal lavages after aspirin intranasal challenge (* $P < 0.05$ placebo vs Lys-ASA challenge, # $P < 0.05$ AIA vs ATA group).

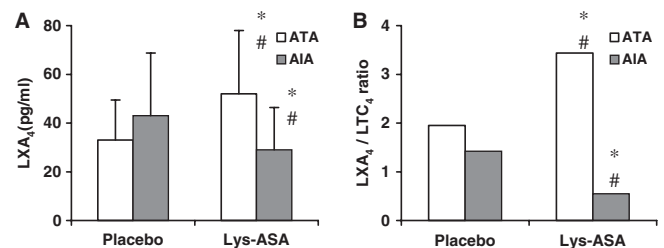


Figure 3. (A) LXA₄ levels (pg/ml) in nasal lavage fluids 2 hours after placebo or Lys-ASA challenge (* $P < 0.05$ placebo day vs. Lys-ASA challenge, # $P < 0.05$ AIA vs ATA group). (B) LXA₄ / LTC₄ ratio in nasal lavage fluids 2 hours after placebo or Lys-ASA challenge $P < 0.05$ placebo day vs Lys-ASA challenge, # $P < 0.05$ AIA vs ATA group).

Table 2. Correlations of LXA₄ production with LTC₄ and eosinophils number

	LXA ₄ /LTC ₄ correlation		LXA ₄ /eosinophils correlation	
	<i>R</i>	<i>P</i>	<i>R</i>	<i>P</i>
Whole group				
Placebo	0.673	0.01	0.516	0.02
Lys-ASA	-0.244	NS	-0.235	NS
AIA group				
Placebo	0.895	0.003	0.549	NS
Lys-ASA	0.467	NS	0.266	NS
ATA group				
Placebo	0.173	NS	0.93	NS
Lys-ASA	-0.058	NS	-0.22	NS

R – two-tailed Pearson correlation, NS – *P* > 0.05.

LTC₄ was significantly reduced in AIA in contrast to ATA study group (Fig. 3B).

In atopic patients suffering from seasonal allergic rhinitis baseline level of LXA₄ was 33.49 ± 16.95 pg/ml and no significant difference was noticed after the clinically positive allergen challenge (36.22 ± 13.26 pg/ml, *P* = 0.23, Wilcoxon's test).

We found a significant correlation between LXA₄ and LTC₄ production in the whole study group after the placebo challenge (*R* = 0.673, *P* = 0.01) and in AIA group after the Lys-ASA challenge (*R* = 0.895, *P* = 0.003). There was a significant correlation between LXA₄ levels and numbers of eosinophils in nasal lavage fluid in the whole group after the placebo challenge (*R* = 0.0516, *P* = 0.02) (Table 2).

As proposed earlier by Sanak et al. (6) the diminished lipoxin production may serve as a useful diagnostic tool to distinguish between AIA and ATA. In our model the value of 40 pg/ml was arbitrary selected as a cut off point for ability to produce LXA₄ after the Lys-ASA challenge. Such a test resulted in sensitivity of 75%, specificity of 75% with 81.8% of positive predictive value (PPV) and 66.67% of negative predictive value (NPV). ROC curve (area under the curve AUC = 0.745, *P* = 0.07, 95% confidence interval 0.498–0.991) is presented on Fig. 4.

Discussion

Nowadays it is widely accepted that once thought to be a passive process, the resolution of inflammation involves active cellular and molecular mechanisms that enable the inflamed tissue to return to homeostasis (3). Endogenous lipid mediators produced by leukocytes such as prostaglandins and leukotrienes activate and amplify inflammation. Several studies showed that within hours after the initiation of inflammation neutrophils stop producing chemoattractants (including leukotriene B₄) and begun to convert arachidonic acid into anti-inflammatory

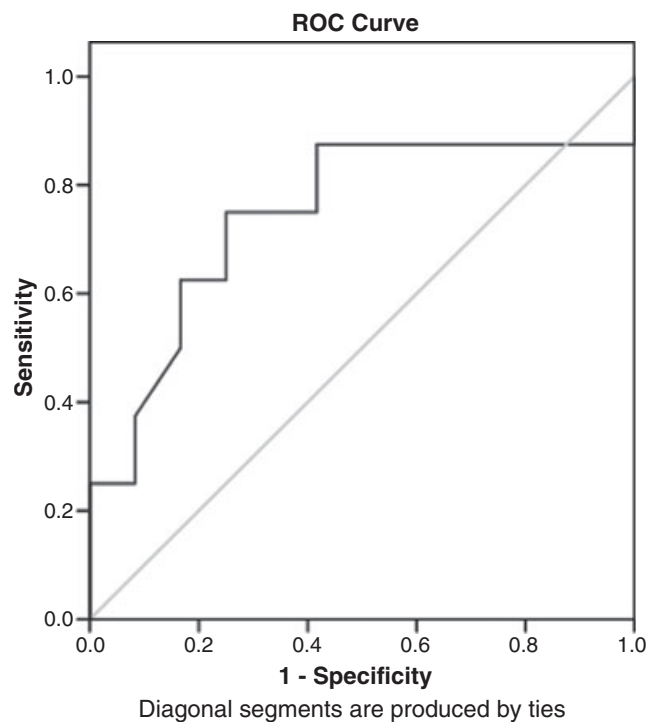


Figure 4. ROC curve with statistical evaluation has been drawn to test the hypothesis of lipoxin production as a diagnostic tool to distinguish between AIA and ATA groups.

mediators, such as lipoxins (3). This process described as lipid-mediator class switching may be partially responsible for the resolution of inflammation and induction of healing processes. Lipoxin A₄ (LXA₄) and lipoxin B₄ (LXB₄) interact with receptors, including a G-protein-coupled specific ALX receptor, that transducer counter-regulatory signals in part via intracellular polyisoprenyl phosphate remodeling (13). Up to date LXs have been identified in many human tissues and their production is clearly decreased in several prolonged and chronic inflammatory diseases (14). Planaguma et al. (4) concluded that defect in lipoxin production and counter-regulatory signaling may be in part responsible for persistent inflammation in severe asthmatics. One can hypothesize that the impairment of a balance between lipoxins' and leukotrienes' production may be of importance in the pathology of the aspirin hypersensitivity.

To our knowledge this paper is the first to demonstrate that aspirin intolerant asthmatics have diminished LXs' biosynthesis capacities *in vivo* after ASA challenge. We have shown that both AIA and ATA patients have detectable levels of LTC₄ and LXA₄ in nasal lavage fluid. Lys-ASA challenge resulted in significant increase of clinical symptoms and influx of inflammatory cells in AIA but not ATA group. In AIA release of LTC₄ reached its maximum 1 hour after the aspirin challenge and was significantly higher in comparison to placebo challenge and both Lys-ASA and placebo challenge in ATA group.

Higher levels of inflammatory cells (eosinophils and basophils) and LTC₄ in samples from AIA compared to ATA patients after placebo challenge confirm well known features of immunopathology of chronic inflammation in the airways of aspirin sensitive subjects. The mean baseline levels of LXA₄ were similar in AIA and ATA groups. Lys-ASA challenge induced a significant increase of LXA₄ only in ATA not in AIA group. In contrast in AIA group we observed a decrease in LXA₄ levels. Moreover if we express the relationship between anti-inflammatory (LXs) and pro-inflammatory (LTs) mediators' synthesis as the ratio of LXA₄ to LTC₄ the observed discrepancies are even more striking. The role of LTs in orchestrating the inflammatory processes in the airways of asthmatic subjects and patients suffering from aspirin sensitivity is well established (15). LXs' role in the pathogenic mechanisms of asthma and AIA may potentially involve interaction in the airway with LTD₄ receptor thus blocking airway smooth muscle contraction, as it has been shown that inhalation of LXA₄ by human asthmatics blocks LTC₄ stimulated bronchoconstriction (16). Moreover lipoxins inhibit eosinophils' trafficking and PMNs' chemotaxis and trans-migration to the site of inflammation. LXs reduce generation of several vital pro-inflammatory cytokines including: eotaxin, TNF, IFN γ , IL1 β , IL-5, IL-6, IL-8, IL-12 and other mediators like metalloproteinase 3 (3). In our study we found that AIA patients have diminished ability to synthesize LXs *in vivo* after exposure to ASA. Lack of LXs may results in impairment of healing processes thus leading to the development of vicious circle of persistent inflammation which may in part explain our clinical observations that aspirin sensitivity syndrome is characterized by chronic inflammation in the airways even in subjects avoiding triggering factors such as ASA and other NSAIDs (17, 18). Clinically positive allergen challenge had no impact on LXs levels in atopic patients. This further confirms fact that LXs are rather involved in resolution of chronic inflammation and are not released on mast cells degranulation during early phase of allergic reaction.

Lys-ASA nasal challenge model and nasal lavage collection have been chosen as well accepted methods both in clinical settings and research studies (8, 11). Moreover one of advantages of nasal lavage technique is that it is easy and safe for patients thus no ethical questions are raised up in contrast to bronchoscopy studies in asthmatic subjects. One of typical features of nasal lavage technique is that the whole procedure is preceded by several saline washings of nasal cavity. Analysis of consecutive samples shows a significant increase of cells' and mediators' levels which mirrors the return of 'washed' to normal, typical for particular subject values. Thus a placebo challenge is required for this method and conclusions should be drawn, as it has been done in our paper, on comparison between the placebo and Lys-ASA challenges in the same time points,

not on changes between baseline and consecutive time points. One limitation of our protocol is that detection of mediators was based only on ELISA methodology and not confirmed with further mass spectrometry analyses. On the other hand several published paper showed that ELISA is a valuable and reproducible method for both LTC₄ and LXA₄ determinations (4, 7). Thus in the latest paper by Planaguma et al. (4) only this method was used. Moreover the LXs' levels found in our nasal lavage samples are in the same range as those reported in bronchoalveolar lavage fluids of asthmatic patients (BAL) (4). As methodologies of BAL and nasal lavage techniques are similar it further confirms consistency of the presented results. It should be borne in mind that levels of both studied mediators are low, close to the kits' detection limits. All unreliable data were excluded from statistical analyses. As corticosteroids have a significant impact on 15-LO, ALX receptor expression and LXA₄ synthesis medications' use was clearly defined in our study group. The nasal steroids' wash out of 2 weeks doubled recommendations of EAACI/GA2LEN guidelines (8) however in further studies longer period (4 weeks) may be more appropriate. LXs levels were determined at 2 hours' time point after the ASA challenge. This time point has been selected upon analysis of LTC₄ changes and a hypothesis that the peak of LTs synthesis may be followed by extensive LXs production as a part of lipid mediators class switching on resolution of inflammation (3, 19). We found a significant correlation between LXA₄ and LTC₄ production in the whole study group and AIA patients after the placebo challenge and between LXA₄ levels and eosinophils' number in the whole group after the placebo challenge. Lack of correlation after Lys-ASA challenge is probably due to discrepancy of observed changes in the levels of LXA₄ in both study groups (increase in ATA but decrease in AIA group). However this hypothesis cannot be further confirmed in the subgroup analysis due to limited number of patients studied (Table 2).

It should be also remembered that LXA₄ kit has a significant (24%) cross-reactivity with 15-epi-lipoxin A₄ thus in our model part of mediators detected may be in fact 15-epi-LXs. To distinguish between LXs and 15-epi-LXs may be important in understanding underlying mechanisms thus should be recommended in further studies and we are looking forward more specific diagnostic tools. However as both epimers act via the same specific receptor (ALXR) and have identical anti-inflammatory properties it may be in our opinion less important from clinical point of view. 15-epimers of LXs (15-epi-LXA₄ and 15-epi-LXB₄) are named 'aspirin-triggered lipoxins' (ATL). Aspirin, in addition to inhibition of COX-catalysed prostanoid formation, by acetylation of COX-2 induces the generation of 15R-HETE (20, 21). 15R-HETE released by epithelial cells in the presence of ASA is transformed by leukocytes' 5-lipoxygenase (LO) into 15-epi-LXs, which have been proven to present

stronger anti-inflammatory properties in comparison to the natural LXs probably due to higher stability (22, 23). Picado et al. found that COX-2 messenger RNA is down expressed in nasal polyps of AIA patients (24) and this has been confirmed by other authors. Sanak et al. were not able to detect 15R-HETE in stimulated blood samples from AIA whereas prominent amounts of 15S-HETE were found (6). Lack of substrate could possibly explain diminished 15-epi-LXs synthesis in AIA. Moreover one could hypothesize that extensive utilization of cellular arachidonic acid resources for LTs synthesis in AIA after exposure to aspirin may also finally results in a lack of substrate for LXs production. In our patients baseline levels of LXs were similar with a nonstatistically significant tendency to higher levels found in AIA group. We could speculate that these differences would be more pronounced if subjects with more severe asthma were included as it was in study by Planaguma (4). As ASA triggers generation of 15-epi-LXs we can conclude that changes observed in our study after Lys-ASA nasal challenge may be in part due to different capacity of AIA and ATA patients to produce 15-epimers of LXs. Our findings are in line with the results of Sanak et al. (6) who showed that blood cells from AIA display a lower biosynthetic capacity to generate both LXs and 15-epi-LXs *in vitro*. Similarly, Kowalski et al. (25) found that unstimulated peripheral blood leukocytes of AIA patients release significantly less LXA₄ (300 ± 70 pg/ml) than those of ATA subjects (690 ± 100 pg/ml). Aspirin added to leukocytes from AIA patients induces dose dependent several-fold increase in production of 15-HETE. Although chiral analyses has not been performed in this study authors deduce that 15-LO and not COX-2 was the source of aspirin induced 15S-HETE rather than 15R-HETE production which may explain the diminished capacity of AIA to produce 15-epi-LXs. This further confirms the hypothesis that activity of 15-LO in AIA patients is controlled by endogenous COX-1 derived prostaglandin E₂ (PGE₂) and blocking of PGE₂ production with ASA results in activation of 15-LO and

15-HETE production (26, 27). However this phenomenon of suppression of PGE₂ biosynthesis by ASA may apply only to local conditions in the airways of patients with AIA as in the latest paper by Mastalerz et al. (28) oral ASA challenge was associated with diminished systemic production of PGE₂ in aspirin tolerant but not in aspirin intolerant subjects. This may also explain discrepancies between our results and the study of Levy et al. (7) who found that oral ASA challenge provoked statistically significant increases of immunoreactive LXA₄ in both aspirin-tolerant and aspirin-sensitive subjects. Other possible explanations may include differences in severity of the diseases between studied subjects and time of sampling after the challenge. It could be hypothesize that nasal and probably also bronchial ASA challenges may have some distinct immunopathology features in comparison to oral ASA exposure. This may find confirmation in clinical outcomes as local in contrast to systemic ASA challenges are usually not associated with the development of the late phase reaction however it could be also simply dose-dependent phenomenon.

As proposed earlier by Sanak et al. (6) the diminished lipoxin production may serve as a diagnostic tool to distinguish between AIA and ATA. In our model arbitrary selected value of 40 pg/ml resulted in acceptable sensitivity and specificity (both 75%) which confirms usefulness of this determination as an additional diagnostic test especially in some doubtful cases. However this hypothesis needs further statistical evaluation in a larger cohort.

In conclusion, our study is the first to confirm that aspirin intolerant asthmatics have diminished LXs' biosynthesis capacities *in vivo* after ASA challenge. Together with overproduction of cysteinyl leukotriens this phenomenon may be in part responsible for impairment of a balance between pro-inflammatory and anti-inflammatory mediator release thus resulting with vicious cycle of chronic and often severe inflammation typical for pathophysiology of aspirin-intolerant asthma.

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