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Fatty Acid Composition of Cultured Fibroblasts Derived from Healthy Nasal Mucosa and Nasal Polyps

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Abstract: Background: Fibroblasts from nasal polyps (NP) of asthma patients have reduced expression of cyclooxygenase 2 (COX-2) and production of prostaglandin E_2 (PGE_2). We hypothesized that the reported alterations are due to alterations in the availability of arachidonic acid (AA). Objective: The objective was to determine the fatty acid composition of airway fibroblasts from healthy subjects and from asthma patients with and without aspirin intolerance. Methods: We analyzed the fatty acid composition of cultured fibroblasts from non-asthmatics (n = 6) and from aspirin-tolerant (n = 6) and aspirin-intolerant asthmatics (n = 6) by gas chromatography-flame ionization detector. Fibroblasts were stimulated with acetyl salicylic acid (ASA). Results: The omega-6 fatty acids dihomo-gamma-linolenic acid (C20:3) and AA (C20:4), and omega-3 fatty acids docosapentaenoic acid (DPA) (C22:5) and docosahexaenoic acid (DHA) (C22:6) were significantly higher in NP fibroblasts than in fibroblasts derived from nasal mucosa. The percentage composition of the fatty acids palmitic acid (C16:0) and palmitoleic acid (C16:1) was significantly higher in fibroblasts from patients with NP and aspirin intolerance than in fibroblasts derived from the nasal NP of aspirin-tolerant patients. ASA did not cause changes in either omega-3 or omega-6 fatty acids. Conclusions. Our data do not support the hypothesis that a reduced production of AA in NP fibroblasts can account for the reported low production of PGE_2 in nasal polyps. Whether the increased proportion of omega-3 fatty acids can contribute to reduced PGE_2 production in nasal polyps by competitively inhibiting COX-2 and reducing the amount of AA available to the COX-2 enzyme remains to be elucidated.

Keywords: arachidonic acid; aspirin intolerance; asthma; cyclooxygenase; eicosanoids; fatty acids; fibroblast; inflammation; nasal mucosa; nasal polyps

1. Introduction

Asthma is a syndrome characterized by the presence of chronic inflammation, resulting in airway obstruction and bronchial hyper-responsiveness that causes wheezing, coughing, and dyspnea [1]. Nasal polyposis (NP) is a chronic inflammatory disease of the sinus mucosa usually seen in association with chronic rhinosinusitis (CRS) and asthma [2]. The pathogenesis of CRS with NP is related to an altered inflammatory state that results in a tissue remodeling process [3].

Aspirin-intolerant asthma (AIA) is a distinct syndrome characterized by asthma, CRS, NP, and aspirin sensitivity. Aspirin sensitivity may be present in 5 to 10% of the asthmatic population [4,5]. The pathophysiological mechanism of AIA is only partially understood and appears to be related to anomalies in the metabolism of arachidonic acid AA [4,5].

AA is produced from membrane phospholipids by the action of phospholipase A2 enzymes; it can then be converted into different eicosanoids. AA can be enzymatically metabolized by three main pathways: P-450 epoxygenase, cycloooxygenases (COXs) and lipoxygenases (LOXs). The LOXs convert AA into leukotriene (LT) A_4, which is the precursor of LTB_4 and cysteinyl leukotrienes (LTC_4, LTD_4 and LTE_4) [6]. The COX pathway produces PGG_2 and PGH_2, which are in turn converted into prostaglandins (PGEs) and thromboxanes. There are two isoforms of the COX enzymes: prostaglandin H synthase-1 (PGHS-1), also known as COX-1, is generally constitutively expressed and is typically considered a "house-keeping" gene; and prostaglandin H synthase-2 (PGHS-2), also known as COX-2, is usually only expressed under inflammatory conditions [7].

Eicosanoids are released from cells during hypersensitivity reactions and are involved in the clinical manifestations of rhinitis and asthma. LTs are potent pro-inflammatory mediators and this can explain why anti-LTs are beneficial in asthma and rhinitis [6]. PGs might act as both pro-inflammatory and anti-inflammatory mediators, depending on the context; this is partly due to the level of expression of the four PG receptors in the cells involved in the response [7]. The COX pathway is the major target for non-steroidal anti-inflammatory drugs (NSAIDs), the most popular medications used to treat fever, pain, and inflammation [7]. However, and in contrast with other inflammatory diseases such as arthritis [7] and cystic fibrosis [8], inhibition of COX with aspirin or NSAIDs does not provide any salutary effect to asthma patients. In fact, for a subset of patients with asthma, ingestion of NSAIDs induces bronchoconstriction and nasal obstruction [4,5]. Interestingly, selective COX-2 inhibitors are usually well tolerated by AIA [4,5].

These observations suggest that eicosanoids can be differentially regulated in asthma, unlike other inflammatory airway diseases [5]. In patients with asthma, and especially in AIA, various data support the existence of an altered regulation of the COX pathway [9–15]. PGE_2 levels have been reported to be low in the nasal polyps of asthma patients, as well as in nasal-polyp and bronchial fibroblasts from asthmatic patients, particularly those with aspirin sensitivity [11,13–15]. As PGE_2 production mostly depends on the level of COX-2 induction under conditions of inflammation, it should be expected that the low production of PGE_2 detected in asthma and nasal polyps would be accompanied by a similar, concomitant alteration in the expression of COX-2. Accordingly, lack of up-regulation of COX-2 in the nasal polyps of asthma patients, both with and without aspirin sensitivity, has been reported in various studies [9–12,15].

The mechanisms involved in the abnormal production of prostaglandin E_2 in nasal polyps, and in particular in those associated with AIA, are still unclear [5]. Various studies have shown that the activity of COX-1 and COX-2 enzymes is controlled differentially by regulating the amount of AA available to the enzymes [6]. As PGE_2 production by COX-1 and COX-2 is dependent, at least in part, on the availability of AA, it could be possible that alterations in the AA supply may account for the anomalies in PGE_2 production reported in nasal polyps and AIA.

A number of observations support the notion that fibroblasts are more than just structural cells with no other physiological or modulator functions [16]. Fibroblasts can contribute to the regulation of inflammatory and immunological responses by producing various growth factors, cytokines and eicosanoids [17].

The hypothesis of the present study establishes that the reported alteration in the production of some prostanoids such as PGE_2 in the nasal polyps of asthma patients, particularly in those with aspirin intolerance, is at least partly due to alterations in the availability of AA.

The main objective of this study is to determine and compare the fatty acid composition of airway fibroblasts from healthy subjects and those from nasal polyps of asthma patients with and without aspirin intolerance.

2. Material and Methods

2.1. Population and Tissue Handling

Nasal polyp tissue was obtained from 12 asthmatic subjects (6 aspirin-tolerant, and 6 aspirin-intolerant) referred to our institution for sinus surgery. The study subjects were selected on the basis of a medical history consistent with severe chronic NP, documented via CT scan [2]. The diagnosis of asthma was established from the clinical history and the demonstration of a reversible bronchial obstruction. Diagnosis of aspirin intolerance was based on a clear-cut history of asthma attacks precipitated by non-steroidal anti-inflammatory drugs (NSAID) and confirmed by aspirin nasal challenge in patients with an isolated episode of NSAID-induced asthma exacerbation [18]. Nasal mucosa from 6 subjects undergoing nasal corrective surgery was used as control. The characteristics of the asthmatic and non-asthmatic patients are shown in Table 1.

Table 1. Characteristics of study subjects.

Variables	Controls	Asthma Aspirin Tolerant	Asthma Aspirin Intolerant
N	6	6	6
Age (years)			
Mean ± SD	53 ± 19	60 ± 16	68 ± 9
Min.–Max.	28–77	44–87	59–82
Gender (M/F)	(4/2)	(5/1)	(1/5)

Age is expressed as mean ± standard deviation (SD).

All patients with NP were on intranasal glucocorticoid therapy that was discontinued at least five days before surgery. None of the patients were on oral GC therapy at the time of surgery, nor had they received any systemic GCs for at least one month prior to surgery. No subjects from the nasal mucosa control group had a history of nasal or sinus disease, nor had they received GCs for any reason. None of the subjects had suffered from an upper respiratory infection during the two weeks prior to surgery.

The subjects were asked for their permission and written informed consent was given to study pathological specimens under a protocol approved by the human investigations committee of our hospital.

2.2. Fibroblast Culture

Specimens obtained during nasal endoscopic surgery were cut into 3 × 3 mm fragments and placed in six-well plates containing Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 100 IU/mL penicillin, 100 µg/mL streptomycin, and 2 µg/mL amphotericin B. After a period of 3 weeks, when fibroblast growth was established, tissue fragments were removed and the first passage was performed. Cultures were washed three times with phosphate buffered saline (PBS) and incubated for 5 min with 0.05% trypsin and 0.02% EDTA. The reaction was stopped by the addition of growth medium, cells were collected by centrifugation (1800 rpm, 5 min), seeded in two 75-cm^2 flasks and grown up to 90% confluence (duplicate per sample). Cells from the sixth passage were used in the study of fibroblasts' lipid composition. When the confluence reached up to 90%, cells were washed with PBS and incubated with DMEM without FBS for 24 h, then the medium was changed for another quantity of DMEM without FBS, for samples stimulated with aspirin; 0.5 mg/dL of aspirin were added, then the samples were left for 24 h in culture before the lipid extraction.

2.3. Lipid Extraction

Total lipids were extracted from fibroblast cells using methanol-chloroform containing 1% BHT according to the method of Bligh and Dyer [19]. The cells were washed three times with ice-cold Ca^{2+}, Mg^{2+}, and free PBS; it was then left and the cells were harvested by scraping. The cells were

pelleted by centrifugation at 1200 rpm at 4 °C for 5 min and the PBS was removed. The cells were washed once with 10 mL ice-cold PBS and centrifuged at 1200 rpm at 4 °C for 5 min, then the PBS was removed. 300 μL of ice-cold PBS was added to the sample, and then an aliquot sample of 50 μL was taken to determine the total protein. The rest of the pellets and PBS were transferred to a glass tube and centrifuged at 2500 rpm at 20 °C for 5 min and the PBS was removed. Two milliliters of distilled water were added, then the sample was sonicated to assure further cell lysis, and then centrifuged at 2500 rpm at 4 °C for 10 min; the supernatant was discarded. The pellets were resuspended by 1 mL of physiological serum then the chloroform-methanol (2:1) were added, then the tube contents were centrifuged and the lower phase was collected and dried under nitrogen. One milliliter of 14% BF3/MeOH reagent was added. The mixture was heated at 100 °C for 1 h. Then it was cooled to room temperature and fatty acid methyl esters (FAMEs) were extracted twice in the hexane phase, following the addition of 1 mL H_2O. The aliquot was evaporated to dryness under nitrogen and re-diluted with 75 μL of n-hexane.

2.4. Analysis of FAMEs

Gas chromatography analyses were performed using an Agilent 7890A system (Agilent Technologies, Barcelona, Spain) equipped with a flame ionization and autosampler. Separation of fatty acid methyl esters was carried out on a SupraWAX-280 capillary column (30 m × 0.25 μm × 0.25 mm I.D.) coated with a stationary phase (polyethylene glycol 100%) from Teknokroma (Barcelona, Spain). The operating conditions were as follows: The split-splitless injector was used in split mode with a split ratio of 1:10. The injection volume of the sample was 1 μL. The injector and detector temperatures were kept at 220 °C and 300 °C, respectively. The temperature program was as follows: initial temperature 120 °C for 1 min., increased at 15 °C/min to 210 °C and held at this temperature for 42 min (total running time: 49 min.). For safety reasons, helium was used as the carrier gas, with a head pressure of 300 kPa that referred to a linear velocity of 27.5 cm/s at 140 °C. Detector gas flows: H_2, 40 mL/min; make-up gas (N_2), 40 mL/min; air, 450 mL/min. Data acquisition processing was performed with HP-chemstation software. Samples were determined in duplicate. The identities of sample methyl ester peaks were determined by comparison of their relative retention times with those of well-known standards. The results are expressed in relative amounts (percentage of total fatty acids).

The same batch of a commercial fetal bovine serum (FBS) was used in all the experiments to prevent any interference of culture procedure in the fatty acid composition of fibroblast. The fatty acid composition of fetal bovine serum was also analyzed.

The protein concentration of the cell lysates was measured using a modified Lowry method, with bovine serum albumin as the protein standard. Absorbencies were read at 630 nm in a spectrophotometer.

2.5. Statistical Analysis

Results were described by means frequencies and percentages for qualitative variables and mean ± Standard Deviation (SD) or Standard Error of mean (SE). Data were analyzed using ANCOVA models, adjusting the effect of studied factor by level of protein. For these inferential analyses, a non-parametrical approach by means rank transformation was applied. Statistical significance was considered at $P < 0.05$. Analyses were carried out using the SPSS program for MS Windows (version 15).

3. Results

3.1. Fatty Acid Composition in Nasal Mucosa and Nasal Polyps

The fatty acid composition of fibroblasts from control nasal mucosa and nasal polyps are shown in Table 2. The omega-6 fatty acids, dihomo-gamma-linolenic acid (C20:3) and AA (C20:4), and the omega-3 fatty acids, DPA (C22:5) and DHA (C22:6), were significantly higher in nasal polyp fibroblasts than in fibroblasts derived from nasal mucosa. In contrast, oleic acid (C18:1), gamma-linolenic acid

(C18:3), and eicosadienoic acid (C20:2) were significantly lower in nasal polyp fibroblasts than in those cultured from nasal mucosa.

Table 2. Fatty acid composition in fibroblasts from the different groups.

	NM	NP	NP-ATA	NP-AIA
	N = 6	*N* = 12	*N* = 6	*N* = 6
16:0	17.34 ± 2.53	17.78 ± 2.96	16.74 ± 3.80	18.83 ± 1.11 [f]
16:1	2.87 ± 0.74	3.02 ± 1.06	2.55 ± 1.17	3.50 ± 0.69 [d]
18:0	16.72 ± 0.95	16.80 ± 1.67	17.25 ± 1.87	16.35 ± 1.36
18:1	33.38 ± 2.77	31.83 ± 2.78 [c]	31.80 ± 3.71	31.86 ± 1.45
18:2n6	2.15 ± 1.14	1.80 ± 0.64	1.60 ± 0.53	2.01 ± 0.70
18:3n6	0.63 ± 0.38	0.40 ± 0.20 [c]	0.36 ± 0.21	0.43 ± 0.19
18:3n3	0.24 ± 0.14	0.18 ± 0.10	0.19 ± 0.10	0.16 ± 0.10
20:2n6	2.36 ± 0.56	1.61 ± 0.51 [a]	1.81 ± 0.45	1.41 ± 0.51 [e]
20:3n6	1.50 ± 0.38	1.73 ± 0.35 [a]	1.64 ± 0.40	1.81 ± 0.26
20:4n6	5.97 ± 2.12	7.20 ± 1.55 [a]	7.39 ± 1.91	7.00 ± 1.10
20:3n3	0.16 ± 0.11	0.18 ± 0.19	0.23 ± 0.26	0.14 ± 0.03
20:5n3	0.66 ± 0.24	0.68 ±0.37	0.76 ±0.40	0.61 ±0.34
22:5n3	1.83 ± 0.40	2.11 ± 0.39 [c]	2.11 ± 0.40	2.11 ± 0.39
22:6n3	2.69 ± 0.72	3.41 ± 1.10 [c]	3.44 ± 1.14	3.38 ± 1.10
Omega 3	5.59 ± 1.09	6.56 ± 1.65	6.73 ± 1.78	6.40 ± 1.54
Omega 6	12.61 ± 3.02	12.73 ± 2.14	12.80 ± 2.67	12.66 ± 1.51
ω6/ω3	2.33 ± 0.68	2.05 ± 0.57	2.00 ± 0.56	2.10 ± 0.60

Values given as mean ± standard deviation (SD); NM, nasal mucosa; NP, nasal polyp; NP-ATA, nasal polyp aspirin-tolerant asthma; NP-AIA, nasal polyp aspirin-intolerant asthma; [a] $P < 0.001$, [b] $P < 0.01$, and [c] $P < 0.05$, as compared to mucosa; [d] $P < 0.005$, [e] $P < 0.02$, and [f] $P < 0.05$, as compared to NP-ATA. The sum of fatty acids percentage composition is not equal to 100%, because there are fatty acids that were analyzed but not mentioned here.

The comparison between the fatty acid composition of fibroblasts from aspirin-tolerant and aspirin-intolerant patients is also shown in Table 2. The percentages of fatty acid composition in palmitic acid (C16:0), and palmitoleic acid (C16:1) were significantly higher in fibroblasts from patients with nasal polyps and aspirin intolerance than in fibroblasts derived from nasal polyps of aspirin-tolerant patients. In contrast, the percentage of eicosadienoic acid (C20:2 n6) was significantly lower in fibroblasts from aspirin-intolerant patients, compared with those cultured from aspirin-tolerant subjects.

3.2. Effects of Aspirin on Fatty Acid Composition

The effect of aspirin on the fatty acid composition of fibroblasts from nasal mucosa and nasal polyps is shown in Table 3. There was only one statistically significant difference in the percentage composition of 20:3n6, between the mean of an induced aspirin fibroblast and its basal status in aspirin-tolerant patients.

The lipid changes in fibroblasts from asthmatic and non-asthmatic patients were accompanied by changes in the amount of total proteins used to normalize the fatty acid composition.

Table 3. Differences in fatty acid composition of fibroblast after being stimulated with aspirin.

	NM	NP	NP-ATA	NP-AIA
	N = 6	*N* = 12	*N* = 6	*N* = 6
16:0	0.03 ± 1.31	−1.44 ± 1.17	−0. 38 ± 2.30	−0.19 ± 0.43
16:1	−0.16 ± 0.46	−0.14 ± 0.38	−0.04 ± 0.62	−0.08 ± 0.43
18:0	−0.46 ± 0.55	0.47 ± 0.63	0.29 ± 0.91	0.27 ± 0.85
18:1	0.83 ± 1.52	0.96 ± 1.13	2.76± 1.83	−0.23 ± 0.90
18:2n6	−0.34 ± 0.70	−0.12 ± 0.24	0.13 ± 0.25	−0.31 ± 0.44

Table 3. *Cont.*

	NM	NP	NP-ATA	NP-AIA
	***N* = 6**	***N* = 12**	***N* = 6**	***N* = 6**
18:3n6	−0.09 ± 0.24	−0.09 ± 0.08	−0.13 ± 0.12	−0.03 ± 0.12
18:3n3	0.06 ± 0.08	−0.04 ± 0.04	−0.02 ± 0.06	−0.08 ± 0.06
20:2n6	0.26 ± 0.34	0.13 ± 0.22	0.36 ± 0.23	−0.03 ± 0.32
20:3n6	−0.16 ± 0.20	0.24 ± 0.13	0.55 ± 0.20 [a]	−0.09 ± 0.14
20:4n6	−0.19 ± 1.07	0.88 ± 0.64	1.66 ± 1.13	−0.16 ± 0.63
20:3n3	−0.11 ± 0.06	−0.09 ± 0.08	−0.18 ± 0.15	0.00 ± 0.02
20:5n3	0.12 ± 0.12	0.07 ± 0.14	0.05 ± 0.23	0.08 ± 0.21
22:5n3	−0.08 ± 0.25	0.12 ± 0.15	0.25 ± 0.20	−0.02 ± 0.24
22:6n3	0.06 ± 0.46	0.67 ± 0.46	0.63 ± 0.68	0.55 ± 0.67
Omega 3	0.05 ± 0.70	0.72 ± 0.67	0.72 ± 1.03	0.53 ± 0.99
Omega 6	−1.24 ± 1.55	1.04 ± 0.87	2.57 ± 1.52	−0.62 ± 0.86
ω6/ω3	0.05 ± 0.07	0.01 ± 0.07	−0.05 ± 0.09	0.07 ± 0.11

Values given as a mean difference of fatty acid composition percentages before and after aspirin stimulation ± standard error of mean (SEM); NM, nasal mucosa; NP, nasal polyp asthmatics; NP-ATA, nasal polyp aspirin-tolerant asthma; NP-AIA, nasal polyp aspirin-intolerant asthma; [a] $p < 0.05$ as compared to the basal status of the same sample before aspirin stimulation.

The fatty lipid composition of fetal bovine serum is shown in Table 4.

Table 4. Total fatty acid composition of the fetal bovine serum used in fibroblast culture.

Fatty Acid	Mean ± SD
14:0	2.45 ± 0.45
14:1	0.39 ± 0.05
16:0	24.97 ± 0.14
16:1	2.81 ± 0.09
18:0	14.04 ± 0.08
18:1n9	23.86 ± 1.03
18:1n7	5.44 ± 0.16
18:2n6	6.74 ± 0.18
18:3n6	0.50 ± 0.04
18:3n3	0.53 ± 0.03
20:0	0.41 ± 0.03
20:1n9	0.75 ± 0.04
20:2n6	0.24 ± 0.02
20:3n6	1.81 ± 0.07
20:4n6	5.31 ± 0.20
20:3n3	0.57 ± 0.01
20:5n3	0.97 ± 0.04
22:0	1.07 ± 0.05
22:1n9	0.57 ± 0.02
22:5n3	0.39 ± 0.01
24:0	1.08 ± 0.12
22:6n3	3.48 ± 0.18
24:1	1.54 ± 0.20

The sample was analyzed in triplicate. Data are listed as mean ± SD.

4. Discussion

The concentration of free AA in resting cells is commonly described as "low". In some inflamed tissues, such as the skin of patients with psoriasis, free AA is abundant, but in healthy skin it is scarcer [20]. Similar results have been reported in fibroblasts from hypertrophic scars as compared to normal dermis [21]. These observations suggest that the level of AA increases under conditions

of inflammation and remodeling, thereby facilitating the subsequent synthesis of PGs, leukotrienes and lipoxins.

We used fibroblasts from nasal polyps to study the impact of inflammation and aspirin intolerance in fatty acid composition, because previous studies have shown very low production of PGE_2 in nasal polyps from both aspirin-tolerant and aspirin-intolerant patients [11,14,15]. Similarly, other studies have also shown a significantly low production of PGE_2 in cultured bronchial fibroblasts from AIA [13].

Previous studies have shown that PGE_2 production in inflammatory situations is directly related to the level of expression of COX-2 [6]. Other studies have also reported that the induction of COX-2 by inflammatory mediators in human lung fibroblasts does not simply result in an increase in all the prostanoids that a given cell can produce. Instead, there is a shift in the balance of PGs toward the preferential production of prostacyclin and PGE_2 [22]. Based on these observations, we reasoned that the altered production of PGE_2 in airway fibroblasts from nasal polyps of asthma patients might be due, at least in part, to an insufficient amount of AA available to the COX-2 enzyme.

Our results do no support this hypothesis. In fact, the percentage of AA present in fibroblasts derived from an inflamed tissue (nasal polyps) was significantly higher that in those cultured from non-inflamed nasal mucosa. These results concur with others that show an increased presence of AA in airway inflammatory processes such as cystic fibrosis, a disease characterized by a chronic inflammatory process affecting both the lower (bronchiectasis) and upper airways (chronic rhinosinusitis and nasal polyps). After performing nasal tissue biopsies, Freedman *et al.* [23] reported significantly higher levels of AA in cystic fibrosis and asthma patients compared with healthy control subjects. Interestingly, Roca-Ferrer *et al.* [9] also found that COX-2 mRNA and protein were markedly up-regulated in NP from cystic fibrosis patients; these findings contrast with the lack of expression of COX-2 mRNA and COX-2 protein in NP from asthma patients. As expected, in cystic fibrosis the increased release of AA, together with the up-regulated COX-2, results in an enhanced production of PGE_2, as has been demonstrated in saliva, exhaled air and urine [24–26].

To our knowledge, only one *ex vivo* study has assessed the fatty acid composition in the cells of asthma patients [27]. In contrast with our study, the authors reported significantly lower levels of AA in platelets isolated from asthma patients, compared with those obtained from health subjects. The reasons for this discrepancy are unclear, although it is most probably explained by the differences in the cells that were used: fibroblasts and platelets. Significantly, COX-2 platelets make a very limited contribution to prostanoid production [28].

Our study's second finding was that, in addition to the increased presence of omega-6 fatty acid AA, there were also higher levels of omega-3 fatty acids DPA (C22:5) and DHA (C22:6) in the fibroblast derived from NP. The membranes of most cells contain large amounts of AA, compared with other potential prostaglandin precursors, including eicosapentaenoic acid (EPA); thus, our finding explains why AA is usually the principal precursor of eicosanoid synthesis, and also why the series-3 prostaglandins (PG_3) that have EPA as their precursor are formed at a slower rate than series-2 prostaglandins (PG_2). These differences usually result in an increased production of omega-6, in contrast with the low formation of omega-3 in inflammatory diseases. When omega-3 exists in high amounts in cells, it can decrease the levels of AA in the membranes of inflammatory cells, so there will be less substrate available for the synthesis of pro-inflammatory eicosanoids [6,29]. In addition, EPA competitively inhibits the oxygenation of AA by cyclooxygenases [30]. Overall, by means of various mechanisms, omega-3 fatty acids can reduce the production of prostanoids such as PGE_2.

Based on these observations, several studies have evaluated the effects of diet manipulation in the treatment of inflammatory diseases, including those such as asthma that affect the airways. The metabolism of PUFA is highly dependent on the availability of lipid precursors. The AA pool for eicosanoids can only be slowly influenced by dietary omega-6 PUFA. In contrast, the omega-3 PUFA pool is usually smaller and can be modified more rapidly by dietary omega-3 PUFA supplementation [31].

Various uncontrolled fish oil trials have shown clinical benefits in asthma. However, a more recent report covering a large number of studies concluded that "no definitive conclusion can yet be drawn regarding the efficacy of omega-3 FA supplementation as a treatment for asthma" [32]. Another review and meta-analysis concluded that it is unlikely that supplementation with omega-3 plays an important role in the prevention of asthma and allergic diseases [33].

Our finding of an increased presence of omega-3 DHA fatty acid in fibroblasts coming from an inflamed nasal tissue contrasts with previous studies showing the opposite effect. Freedman *et al.* [23], and Javier de Castro *et al.* [27] reported very low proportion of DHA in the nasal tissue of cystic fibrosis patients and platelets of asthma patients, respectively. Differences in the studied disease (cystic fibrosis *vs.* asthma) and in the cells (fibroblast *vs.* platelets) most probably account for the discrepancies.

Recent studies support the notion that PGE_2 exerts anti-inflammatory rather than pro-inflammatory actions in the lung [34]. We are tempted to speculate that the increased production of omega-3 in nasal polyps competitively inhibits the oxygenation of COX-2 and results in a reduced production of the anti-inflammatory PGE_2. If this hypothesis true, any attempt to increase the amount of cell-membrane omega-3 fatty acids by increasing the dietary intake of EPA would result in deleterious rather than salutary effects in asthma patients, particularly in AIA.

Interestingly enough, a diet supplemented with fish oil for 6 weeks was associated with a reduction in prostanoid production, a mild clinical deterioration and increased bronchial obstruction in a group of aspirin-intolerant asthma patients [35]. This observation concurs with a recent study reporting that fish oil supplementation in a mouse model of asthma led to a significant suppression of the production of PGE_2, associated with both an enhanced lung inflammatory response and an increased release of pro-inflammatory cytokine IL-5 and IL-13 [36]. All in all, these results bring into question the proposed potential protective role of fish oil supplementation in the treatment of airway inflammatory processes such as asthma and chronic rhinosinusitis with nasal polyps.

Aspirin-intolerant asthma is characterized by a persistently elevated production of cysteinyl leukotrienes (Cys-LTs) in a steady state, due to the up-regulation of the 5-lipoxygenase pathway enzymes [4]. The release of Cys-LTs is further enhanced when these patients are challenged with aspirin and suffer an episode of bronchoconstriction [4]. The mechanism responsible for the increased release of Cys-LTs is only partially known, although the inhibition of COX-1 appears to be the crucial precipitating event [4,5]. In contrast, selective COX-2 inhibitors are usually well tolerated, do not increase the release of Cys-LTs, and do not cause bronchoconstriction [4]. Diversion of AA from the COX-1 pathway to the 5-LO pathway has been suggested as an explanation for the increased release of Cys-LTs after aspirin exposure [4]. Whether this AA diversion is also accompanied by an increase in the release of AA from membrane phospholipids is a possibility that has not yet been examined. We assessed the effects of aspirin on fatty acid composition in fibroblasts from aspirin-tolerant and aspirin intolerant patients and could not find any change in the levels of AA. This finding suggests that aspirin does not enhance the release of 5-lipoxygenase metabolites by increasing the amount of AA available to the lipooxygenase enzymes.

We compared the fatty acid composition of fibroblasts derived from NP of aspirin-tolerant and aspirin-intolerant patients and we found an increase in the percentage composition of palmitic acid (C16:0). The significance of this difference remains to be clarified.

In summary, the objective of this study was to investigate whether the previously reported low release of PGE_2 in fibroblasts from asthma patients could be caused by a reduced supply of AA. Our data shows an increased presence of the omega-6 AA and the omega-3 DPA and DHA fatty acids in nasal polyp fibroblasts compared with nasal mucosa fibroblasts. Whether the increased presence of omega-3 fatty acids can contribute to reducing PGE_2 production in nasal polyps by competitively inhibiting COX-2 and reducing the levels of AA available to the COX-2 enzyme in the membranes of the inflammatory and structural cells remains to be elucidated.

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