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n-3 Fatty acids as resolvents of inflammation in the A549 cells

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ABSTRACT

Background: Fatty acids and their derivatives are one of the most crucial inflammation mediators. The aim of our study was to evaluate the impact of polyunsaturated fatty acids as eicosanoids precursors on the A549 cell line.
Methods: Cells were incubated with 40 μM of arachidonic, eicosapentaenoic or docosahexaenoic acid for 24 h, then activated with LPS. Fatty acids content in the cell membranes were determined using gas chromatography. COX-2, cPGES and FP-receptor quantities were determined by Western blot. 8-Isoprostane F2α concentrations were determined by EIA. Maresin and protectin D1 contents were analyzed by UHPLC/MS-TOF method.
Results: Significant differences in membrane fatty acids and levels of 8-isoPGF2α in the activated cells were detected. Elevated expression of COX-2 and FP-receptor was observed in cells treated with AA and activated with LPS. Moreover, compared to AA and AA + LPS groups, cells incubated with EPA, DHA, EPA + LPS and DHA + LPS showed decreased expression of COX-2, cPGES and FP-receptor. In cells incubated with EPA or DHA and activated with LPS maresin and protectin D1 were detected.
Conclusions: The results of the study have revealed the pro-inflammatory properties of AA, while the EPA and DHA had the opposite, resolving effect. Interestingly, FP-receptor inhibition by EPA and DHA demonstrated the unique role of the FP-receptor as a potential target for antagonists, in the diseases of inflammatory character. This study provides new information about n-3 fatty acids and their pro-resolving mediators, which can be used in the process of developing new anti-inflammatory drugs.

Introduction

Eicosanoids are one of the most crucial inflammation mediators. Their pro-inflammatory properties were well known, however, current studies focus on the anti-inflammatory effect of fatty acids derivatives.

The basic physiological mechanisms that may regulate the inflammatory process, which is genetically determined, include the endogenous eicosanoids system and apoptosis. Inflammation is a defensive reaction of living tissues to injury. Acute inflammation may go into a chronic form, with adverse health consequences in the form of difficult-to-treat chronic diseases. Studies of the mediators of inflammation arising from fatty acids led to the identification of the anti-inflammatory potential of polyunsaturated fatty acids (PUFAs). These lipid mediators exert their anti-inflammatory effects in a receptor-dependent manner. The most important effects contributing to the inflammatory response are: inhibition of neutrophil mobilization and transmigration, suppression of pro-inflammatory cytokine secretion by various cells present in the region of

Abbreviations: AA, arachidonic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; LPS, lipopolysaccharide; 8-isoPGF2α, 8-iso prostaglandin F2α; PTGS2, prostaglandin synthase 2; COX-2, cyclooxygenase-2; cPGES, cytosolic prostaglandin E synthase 3; FP-receptor, prostaglandin F2α receptor; 7(R)-MaR1, 7(R)-Maresin; protectin DX, 10(S),17(S)-DiHDoHE, protectin D1.

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inflammation, and the stimulation of the phagocytic activity of monocytes and macrophages. The molecular mechanism to resolve the inflammatory response by lipid mediators is not fully elucidated [1–4].

Lipids are essential molecules for the structure of cells and organelles. Cellular membranes require specific structural motifs for optimum functionality both under physiological conditions and to withstand cellular stresses. Thus, complex metabolic pathways are needed to properly synthesize and degrade membranes, depending on the needs of the cell. However, in multicellular organisms, a large fraction of the organism's effort must be spent on intercellular communication, so that different cells and tissues may function in synchrony [5–8].

Understanding the immune–metabolic interface is daunting. One contemporary issue is characterizing the sources and mediators of chronic low-level metabolic inflammation, which were first characterized in adipose tissue, but are now known to be present in many different tissues including the liver, muscle, pancreas and hypothalamus. A greater understanding of the critical nodes of immune-metabolism may provide opportunities to break the tight connection of defects in metabolism and immunity that propagate disease [9–16].

The dense clustering of different cell types during inflammation presents a unique situation for how lipids are handled. In contrast to the synthesis of protein mediators (cytokines), lipid mediators can be produced along enzymatic pathways that involve multiple cells, in a process known as trans-cellular biosynthesis. In essence, the inflamed tissue becomes a specialized organ for lipid metabolism, producing the types and amounts of lipid mediators needed to promote or resolve inflammation [1–4].

The aim of this study was to evaluate the pro- or anti-inflammatory impact of n-3 and n-6 PUFAs as eicosanoid precursors on COX-2, cPGES and FP-receptor expression and on the 8-isoPGF2a, maresin and protectin D1 formation in the human lung epithelial cancer A549 cells activated with LPS.

Materials and methods

Reagents

8-iPGF2α, 8iPGF2α-d4, 10(S),17(S)-DiHDoHE, 7(R)-Maresin were obtained from Cayman Chemical Company (Michigan, USA). LC–MS grade methanol, BHT, AA, EPA and DHA, KOH, were purchased from Sigma–Aldrich (St. Louis, USA). Formic acid was supplied by Merck (Darmstadt, Germany).

Cell cultures

Human Lung Carcinoma Epithelial Cells (A549, ATCC CCL-185) were cultured in F 12K Medium (ATCC) with 10% Fetal Bovine Serum (ATCC, LGC Standards, Poland) and the following antibiotics: penicillin (100 IU/ml) and streptomycin (100 μg/ml) (ATCC). Cells were maintained at 37 °C in a humidified atmosphere which contained 5% CO_2 and were seeded into a 6-well plate (Sarsted, Germany) at a density of 5×10^5 cells/well in 2 ml of medium. During every step of the procedure, cell morphology was investigated by an inverted light microscope (Olympus, Japan). Cell viability during culturing was assessed using the Trypan Blue Exclusion Test.

A549 cells were incubated with 4 μl of arachidonic, eicosapentaenoic or docosahexaenoic acid (AA, EPA and DHA dissolved in ethanol to a final concentration of 10 μM/μl) for 24 h combined with 1 μl of LPS dissolved in PBS to a final concentration of 10 ng/μl for 24 h. Control cultures received the same concentration of ethanol (the final content did not exceed 0.12%, v/v) and PBS as experimental cells.

ApoTox-Glo Triplex Assay

The ApoTox-Glo Triplex Assay was performed to assess cell viability, the potential cytotoxicity of AA, EPA, DHA and LPS, as well as cell apoptosis, as per manufacturer instructions (Promega, MI, USA). Absorbance, fluorescence and luminescence were measured using the FLUOstar Omega plate reader (BMG LABTECH, Germany).

Fatty acid analysis

Cell membranes were prepared by hypotonic hemolysis at 4 °C in 10 mM Tris with pH = 7.4. Membranes were then isolated by centrifugation (10,000 × *g*, 15 min) according to the method proposed by Graham [17].

Total lipid extraction from the cell membranes was carried out using a solution of chloroform/methanol (2:1) [18]. The synthesis of fatty acid methyl esters from the membranes was carried out with 14% BF_3 in methanol [19]. The FA methyl esters were analyzed using gas chromatography (Agilent 6890N) with a DB-23 (60 m, 0.25 μm) column as described earlier [20]. Fatty acid methyl esters were identified according to standards (Sigma–Aldrich). The data were analyzed using ChemStation. Results were expressed in relative percentage of the sum of saturated (SAT), unsaturated (UNSAT), monounsaturated (MUFA), n-3 and n-6 FA.

8-Isoprostane Express EIA Kit

In the cell samples after SPE extraction 8-isoPGF2α concentrations were detected with 8 Isoprostane Express EIA Kit, according to the manufacturer instructions (Cayman Chemical). Absorbance was measured at the FLUOstar Omega plate reader (BMG LABTECH, Germany).

UHPLC/MS-TOF method

Identification and determination of 7(R)-Maresin 1, 10(S),17(S)-DiHDoHE was performed using an UltiMate 3000 RS liquid chromatography system (Dionex, USA) coupled to a mass spectrometer with a time of flight mass analyzer (MicroTOF-Q II, Bruker, Germany). Separation of the studied eicosanoids was carried out on a Synergi 4μ Hydro-RP 80A column (150 mm × 2.0 mm I.D., Phenomenex, USA) at 40 °C. The mobile phase was prepared by mixing methanol and 0.01% formic acid according to a gradient program: MeOH: 0 min – 20%; 18 min – 100%, 20–22 min – 20%, flow 0.2 ml/min. The $[M-H]^-$ masses with the defined retention time (Rt) were: for the 17(S)-DiHDoHE $m/z = 359.222 \pm 0.005$ (Rt = 19.83), for 7(R)-Maresin 1 $m/z = 359.222 \pm 0.005$ (Rt = 20.03 min). The details and validation parameters of the method were described earlier [21].

Western blot for COX-2, cPGES and FP-receptor quantities

Cell lysates were prepared using Mammalian Protein Extraction Reagent (M-PER, Thermo Scientific, Rockford, IL, USA) with protease inhibitor cocktail set III (Calbiochem, Merck, Germany). Protein concentrations were determined using the Bradford reaction. Aliquots (20 μg of protein) were solubilized in a Laemmli buffer with 2% 2-mercaptoethanol (Bio-Rad, Hercules, CA, USA) and subjected to 10% SDS–polyacrylamide gel electrophoresis. Protein extracts were transferred to PVDF membranes using a trans-blot (Bio-Rad). Following transfer, membranes were blocked in the presence of casein in TBS-1% Tween buffer (Bio-Rad) and subsequently incubated either with COX-2, cPEGS primary antibodies diluted 1:1000 in Signal+ for Western blot (GeneTex, Irvine, CA, USA), FP-receptor primary antibody (Cayman Chemical) diluted 1:500 in Signal+ (GeneTex) and β-actin diluted 1:1000 in

Table 1
Fatty acid profiles of the membranes of A549 cells. Data are expressed as [%]. Means ± SD, $n = 6$.

	Control	LPS	AA	AA + LPS	EPA	EPA + LPS	DHA	DHA + LPS	p
SAT	40.8 ± 3.2	35.1 ± 2.5*	34.5 ± 2.4*	33.3 ± 3.1*	40.0 ± 3.5	45.4 ± 1.2**	46.1 ± 2.5**	46.4 ± 3.2**	0.05
UNSAT	59.2 ± 2.9	64.9 ± 3.5*	65.5 ± 3.8*	66.7 ± 2.7*	60.0 ± 2.2*	54.6 ± 2.0	53.9 ± 3.0	53.6 ± 4.0	0.05
MUFA	25.3 ± 1.7	23.7 ± 1.2	23.8 ± 1.5	17.2 ± 1.1*	21.2 ± 0.8	31.7 ± 2.6**	22.7 ± 2.4	30.7 ± 3.6**	0.05
n-3	4.4 ± 0.5	2.7 ± 0.2*	2.9 ± 0.4*	4.0 ± 0.6	25.1 ± 1.3**	9.8 ± 0.8***	17.8 ± 1.1**	10.6 ± 2.2***	0.04
n-6	29.5 ± 1.4	38.5 ± 2.8*	38.8 ± 2.5*	45.5 ± 3.1*	13.7 ± 1.2**	13.1 ± 2.4*	13.4 ± 1.4**	12.3 ± 2.6**	0.03
n-3/n-6	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	1.8 ± 0.4	0.7 ± 0.1**	1.3 ± 0.1*	0.6 ± 0.1**	0.01

SAT, sum of saturated FAs; UNSAT, sum of unsaturated FAs; MUFA, sum of mono-unsaturated FAs; n-3, sum of n-3 FAs; n-6, sum of n-6 FAs; n-3/n-6, n-3/n-6 FAs ratio. *, **, *** – asterisks denoted statistical significant differences between groups.

Signal+ (GeneTex). After washing, the membranes were incubated with Easy Blot anti-rabbit IgG (HRP) as secondary antibodies (GeneTex). The membranes were washed, and proteins were detected using a Clarity Western ECL Luminol Substrate (Bio-Rad) Western blot detection kit. The integrated optical density of the bands was quantified using Chemi Doc Camera with Image Lab software (Bio-Rad).

Statistical analysis

All experiments were performed six times. All data are presented as means ± SD. Differences between groups were determined using the one-way ANOVA, or the Kruskal–Wallis test if normality was not observed. Calculations were performed using Statistica 10 (StatSoft, Poland) software, and statistical significance was defined as $p \leq 0.05$.

Results

Cells viability

No cytotoxic effects were observed in the A549 cells treated with AA, EPA or DHA for 24 h and activated with LPS for 24 h. Caspase-3/7 activity increased in the cells treated with LPS and AA + LPS. Cells viability decreased from 99% to 95% after incubation with LPS for 24 h. There were no apoptotic cells after treatment with these compounds.

Fatty acids content

In the A549 cells we observed differences in the content of membrane fatty acids between all experimental groups. Control cells had 40.8% SAT and 59.2% UNSAT FA. After activation with LPS increases in UNSAT FA were observed ($p \leq 0.05$). Cells treated with EPA and LPS and with DHA and LPS had decreased UNSAT indices compared to controls. In the group supplemented with AA and activated with LPS the MUFA index was the lowest (17.2%) compared to other groups while in the cells incubated with EPA and LPS the MUFA index was the highest (31.7%) ($p \leq 0.05$). The highest concentration of n-6 FA was observed in the AA + LPS samples (45.5%); the lowest amount of n-6 FAs were observed in the A549 cells incubated with EPA, DHA and EPA + LPS. We observed a decreased amount of n-3 FAs in all groups treated with LPS (Table 1).

8-isoPGF2α, maresin and protectin D1 content

The highest amount of 8-iso prostaglandin F2α was detected in A549 cells activated with LPS (1.45 ng/ml) and in the cells supplemented with AA and activated with LPS (1.62 ng/ml) ($p \leq 0.05$). For the cells incubated with AA the content of 8-isoPGF2α was lower (0.76 ng/ml), but differ statistically between others groups ($p \leq 0.05$). In the cells supplemented with EPA or DHA and activated with LPS we observed the same level of 8-isoPGF2α (0.22 ng/ml) which did not differ statistically between control, EPA or DHA cells (Fig. 1).

After UHPLC/MS-TOF analysis we detected maresin and protectin D1 only in the cells supplemented with EPA or DHA and activated with LPS. The content of maresin was 1.58 ng/ml, and PDX concentration was 1.56 ng/ml in the EPA + LPS group. In the DHA + LPS group we observed higher content of maresin (1.70 ng/ml) and protectin D1 (1.67 ng/ml) (Figs. 2 and 3).

COX-2, cPGES and FP-receptor expression

The highest amount of COX-2 compared to control was observed in A549 cells activated with LPS and incubated with AA + LPS. Also higher expression of this protein was observed in the

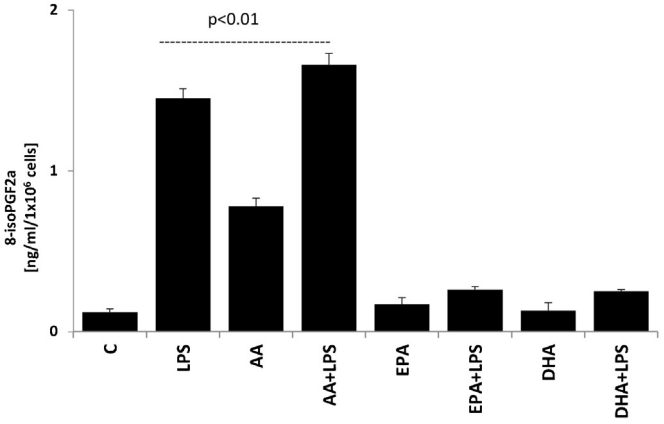

Fig. 1. 8-iso prostaglandin F2α concentration [ng/ml] in A549 cells incubated with AA or EPA and activated with LPS. Means ± SD, $n = 6$.

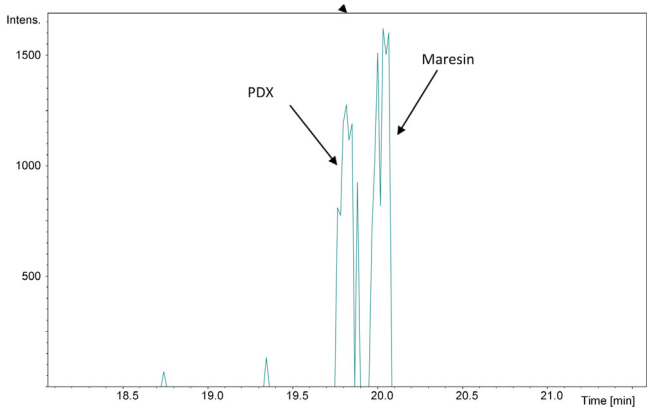

Fig. 2. Chromatogram of maresin and protectin D1.

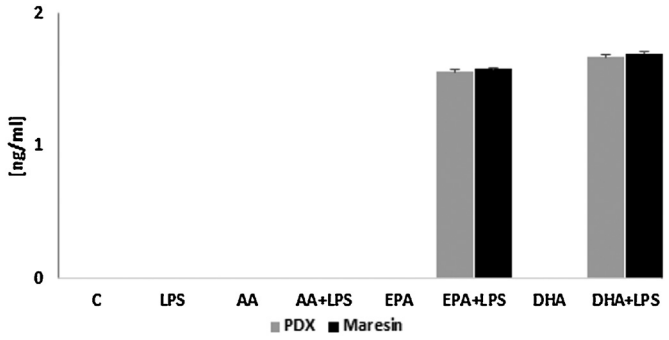

Fig. 3. Maresin and protectin D1 content [ng/ml] in A549 cells supplemented with EPA or DHA and activated with LPS, $n = 6$.

cells supplemented with AA. COX-2 expression was at the control level in the samples incubated with EPA, DHA, EPA + LPS and DHA + LPS compared to the other groups (Fig. 4).

The highest amount of prostaglandin E synthase was observed in the control, AA + LPS and DHA cells. In samples incubated with LPS, AA, EPA, DHA + LPS the level of protein was lower. Supplementation with EPA + LPS caused decreased expression of cPGES compared to the other groups (Fig. 4).

The highest expression of FP-receptor was observed in cells activated with LPS and in the cells AA + LPS. Also high amount of this protein was observed in cells incubated with AA compared to control, EPA and DHA samples. In the cells from EPA + LPS and DHA + LPS groups expression of FP-receptor was higher (Fig. 4).

Discussion

In the present study human lung epithelial carcinoma cells (A549) were incubated with arachidonic, eicosapentaenoic or docosahexaenoic acids for 24 h. Then, cells were activated with LPS for 24 h. A549 cells could synthesize lecithin with a high percentage of desaturated FAs using the cytidine diphosphocholine pathway, so they are an excellent choice to study lipid interactions, metabolism, and signaling [22,23].

Our study is the first of its kind in that it evaluated the FA profile of A549 cell membranes activated with LPS, 8-isoPGF2α levels, maresin and protectin DX content and COX-2, cPGES and FP-receptor activity together.

Our major findings are as follows: (1) supplementation of A549 cells with AA, EPA, DHA or LPS had no effect on viability or apoptosis; (2) there were differences in the content of membrane fatty acids upon activation with LPS; (3) cells incubated with EPA and DHA and activated with LPS had a decrease in cyclooxygenase, prostaglandin E synthase and FP-receptor expression, compared to AA + LPS cells; (4) the level of 8-isoPGF2α after incubation with EPA + LPS and DHA + LPS was significantly lower compared to cells treated with AA and LPS, moreover in both groups maresin and protectin D1 were detected, which may be due to the antioxidant and pro-resolving properties of n-3 fatty acids. n-3 PUFAs exert anti-inflammatory actions by inhibiting pro-inflammatory signal transduction pathways whereas n-6 PUFAs facilitate inflammation by serving as a precursor of pro-inflammatory eicosanoids [7,9,12]. n-3 and n-6 FAs are ligands/modulators for the nuclear receptors NF-κB, PPARs and SREBP-1c, which control various genes of inflammatory signaling and lipid metabolism. n-3 FAs down-regulate inflammatory genes and lipid synthesis, and stimulate FA degradation. In addition, the n-3/n-6 PUFA content

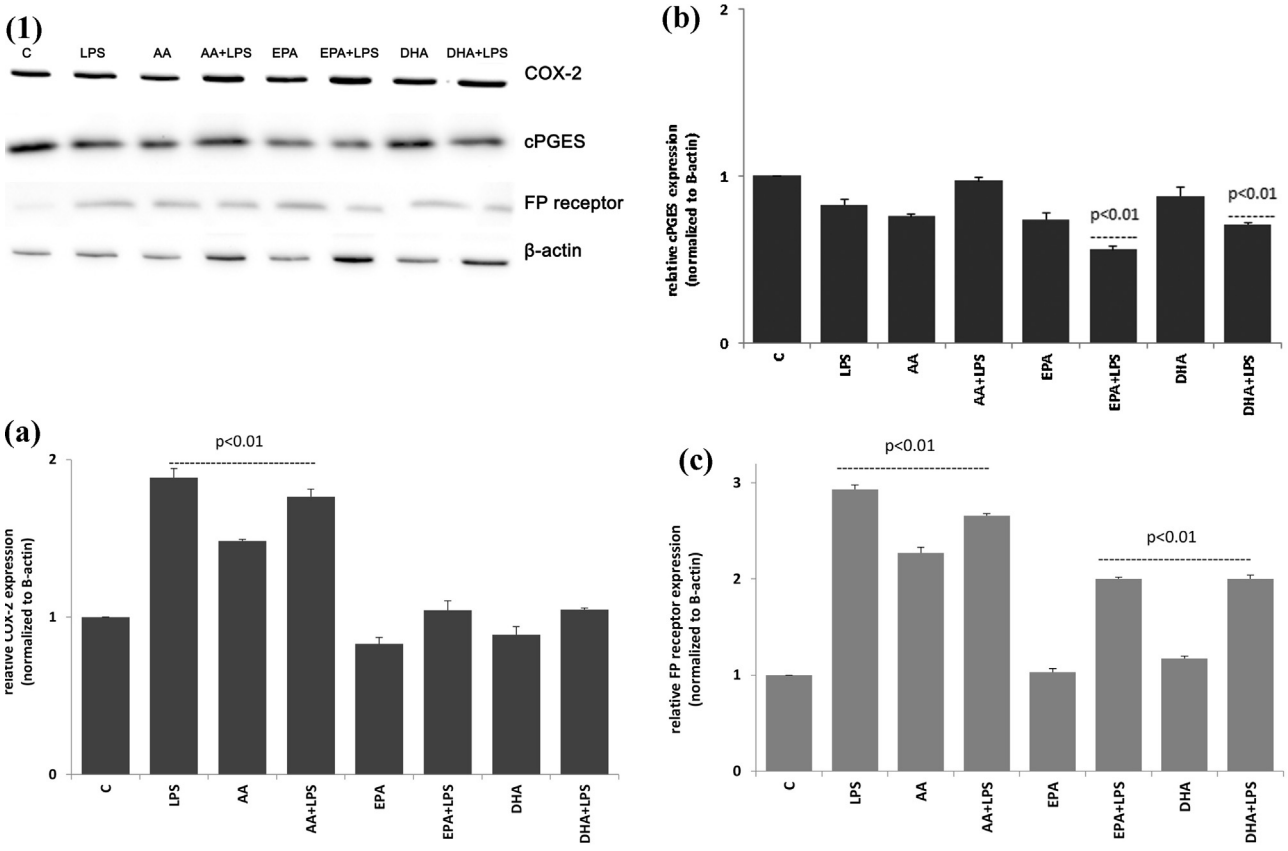

Fig. 4. Relative expression of (a) COX-2, (b) cPGES and (c) FP-receptor in A549 cells incubated with AA or EPA and activated with LPS.

of cell and organelle membranes, as well as membrane microdomains strongly influence membrane function and numerous cellular processes such as cell death and survival [1,24–27].

Mickleborough et al. determined which of the active constituents of fish oil, EPA or DHA, is most effective in suppressing pro-inflammatory mediator generation and cytokine expression from LPS-stimulated human asthmatic alveolar macrophages. The authors showed for the first time that EPA is a more potent inhibitor of inflammatory responses in human asthmatic AMphi cells than DHA [28]. Saedisomeolia et al. determined that EPA and DHA reduced the release of inflammatory mediators from airway epithelial cells infected with rhinovirus (RV). Authors observed that cellular DHA content negatively correlated with IL-6 and IP-10 release. It was concluded that DHA has a potential role in suppressing RV-induced airway inflammation [29].

The results of our study have revealed the pro-inflammatory properties of AA, while the EPA and DHA supplementation had the opposite, anti-inflammatory, resolving effect on the A549 cells. Our data are similar to results observed by Saw et al. [30]. Authors examined whether curcumin with DHA or EPA would have synergistic anti-inflammatory effects in RAW 264.7 cells and showed the synergistic anti-inflammatory as well as anti-oxidative stress effects of curcumin and PUFAs [30]. Wang et al. investigated the role of Nrf2 in suppressing LPS-mediated inflammation in *ex vivo* macrophages by the DHA and EPA. Researchers observed that DHA and EPA inhibited LPS-induced COX-2, iNOS, IL-1β, IL-6, or TNF-α, but increased hemeoxygenase (HO-1) expression [5]. Our studies on cells activation with LPS and supplemented with n-3 FAs confirmed this observation.

In our study, cells incubated with EPA, EPA with LPS, DHA and DHA with LPS had a decrease in cyclooxygenase and FP-receptor expression compared to the AA and AA + LPS groups, which demonstrates the anti-inflammatory properties of the EPA and DHA fatty acids. Interestingly, FP-receptor inhibition by EPA and DHA or their derivatives demonstrated the unique role of the FP-receptor as a potential target for antagonist in the diseases of inflammatory character. Because COX-2 is a key enzyme in the inflammatory response, increasing the expression of this protein by some compounds may be regarded as a pro-inflammatory characteristic. In lung epithelial cells, the pro-inflammatory actions of AA were very pronounced in cells incubated with AA, both with and without LPS activation. The samples of A549 cells incubated with EPA showed decreased COX-2 activity.

The formation of PGE2 from PGH2, by COX-1 or COX-2 is catalyzed by isomerases, three different PGE synthases: cPGES, and two membrane-bound PGE synthases: mPGES-1 and mPGES-2. In immediate PG biosynthesis, which occurs within several minutes after stimulation with agonists that increase cytoplasmic Ca^{2+} levels, cytosolic phospholipase A2 (cPLA2) is a prerequisite for supplying AA to the COX-1. Delayed PG biosynthesis, which proceeds gradually over a long term period after a pro-inflammatory stimulus, is accompanied by *de novo* induction of COX-2, which is an absolute requirement irrespective of the coexistence of COX-1. When cells are first treated with pro-inflammatory stimuli and subsequently exposed to Ca^{2+} mobilizers, the inducible COX-2 can also promote an immediate response [25,26,31,32].

In our study we observed that supplementation of cells with EPA and LPS caused decreased expression of cPGES, compared to the other groups. These results confirmed by the results received for other authors, suggested pro-resolving action of eicosapentaenoic acid.

Wortman et al. showed that EPA-mediated inhibition of AA metabolism did not significantly alter fatty acid synthase (FAS) activity, while both AA and EPA significantly decreased FAS mRNA expression. COX-2 inhibition significantly decreased PGE_2 production resulting in a decrease in FAS activity and expression suggesting an additional mechanism that is independent of COX-2 [33]. In the study by Li et al., DHA pretreatment for 24 h attenuated macrophage activation primarily *via* inhibition of COX-2 induction and activity induced by LPS, with no effects on COX-1. These effects were different from EPA's actions, which lead to inhibition of PG biosynthesis by dampening COX-1. Authors observed that DHA is a more potent inhibitor of COX-2 than COX-1 in terms of PG biosynthesis. Moreover, they showed that DHA also suppresses mPGES-1 expression with prolonged stimulation [7].

EPA competes with AA for release from membrane phospholipids and for metabolism to eicosanoids by COX and LOX. Some studies demonstrated that systemic EPA supplementation can significantly reduce the levels of the key pro-inflammatory mediators PGE2 and 12-HETE relative to their EPA-derived counterparts PGE3 and 12-HEPE, which may have important consequences for regulation of responses to inflammatory stimuli [4,8,34].

Not only can EPA replace arachidonic acid in phospholipid bilayers but is also a competitive inhibitor of cyclooxygenase. Some studies suggest that the beneficial effects of n-3 FAs may be mediated, in part, by the anti-inflammatory effects of oxidized EPA [11,27,35].

Endogenous mediators of inflammation and mechanisms involved in regulating this process, as well as, its resolution are of wide interest, and thus their tissue signature profiles may be useful in characterizing health and disease states, as well as in monitoring the design and impact of new treatments [1,2]. New class of mediator now comprises several new families enzymatically derived from n-3 PUFAs, including E- and D-series resolvins, (neuro)protectins, and maresins [3,4,9].

The antioxidant properties n-3 FAs and its pro-resolving properties were observed in our study. In the A549 cells supplemented with EPA or DHA and activated with LPS, maresin and protectin D1 were detected.

Isoprostanes are good markers of lipid peroxidation; they are formed by a free-radical attack on AA localized in cellular membranes. Currently, 8-iso prostaglandin F2α is the best characterized and the most often studied isoprostane. Separate evidence has suggested that cyclooxygenase activity may also contribute to isoprostane production in selected tissues [4,36].

After analysis we observed that 8-isoPGF2α concentrations were the higher in A549 cells incubated with LPS and in AA + LPS. The level of 8-isoPGF2α after incubation with EPA or DHA and LPS was significantly lower. These results are comparable to those obtained by Gdula-Argasińska et al. [21]. Researchers used human HepG2 cells and estimated the effect of supplementation with EPA and treatment of benzo(a)pyrene. Analysis showed the presence of lipid derivatives of EPA – PGF3α, 8-iPGF3α, and AA derivatives. Authors demonstrated that EPA has an anti-oxidative stress effect under benzo(a)pyrene exposition. Their findings strongly suggest that EPA plays a role in the enhancement of anti-oxidant defense [21].

Changing the FA composition of the membrane cells also affects their properties, function, and cell signaling [37,38]. These effects appear to mediated at the membrane level suggesting the important roles that FAs play in membrane order, lipid raft structure and function, and membrane trafficking.

In the presented study there were significant changes in the FA profile in all groups of cells, those LPS-activated and those supplemented with AA or EPA in relation to the control group. It was found that the LPS-activated cells displayed significantly increased n-6 FAs, with the exception of LPS-activated A549 cells and supplemented with EPA or DHA. Differences in the membrane FA profiles of cells may result from the use of FA for biosynthesis of eicosanoids and from differential expression of genes involved in

the synthesis, elongation, and desaturation of FA at the inflammatory conditions. Therefore enzymes that regulate FA metabolism are attractive candidates to better understand the relationship between FA and inflammation [25,33,37,39]. Stearoyl-CoA desaturase 1 (SCD1) is rate limiting for the conversion of saturated FA to monounsaturated FA. Evidence suggests that SCD1 activity may be positively associated with inflammation. Moreover, genetic variation in SCD1 may alter enzyme activity; however, it is unknown whether this affects inflammatory status [37].

Fatty acids and lipid metabolites that are ligands for PPARs and NF-κB transcription factors are likely to be used as nutraceuticals in the regulation of the immune response.

In conclusion, the results of the study have revealed the pro-inflammatory properties of AA, while the EPA and DHA had the opposite, resolving effect. Interestingly, FP-receptor inhibition by EPA and DHA demonstrated the unique role of the FP-receptor as a potential target for antagonists, in the diseases of inflammatory character. This study provides new information about n-3 FAs and their pro-resolving mediators, which can be used in the process of developing new anti-inflammatory drugs.

Conflict of interest

The authors disclose no other conflicts of interest.

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