

Identification of lipid derivatives in Hep G2 cells*

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Metabolism of polyunsaturated fatty acids results in biosynthesis of mediators with different physiological effects. These metabolites include prostaglandins, prostacyclins, isoprostanes and others that are important signalling molecules and regulate a variety of physiological and pathophysiological processes including inflammation. Prostaglandins and isoprostanes are produced by either non-enzymatic lipid peroxidation or by enzyme-induced peroxidation (cyclooxygenases and lipoxygenases). They are used as biomarkers of oxidative stress. The aim of our study was to assess the effect of eicosapentaenoic acid (EPA) supplementation with added benzo(a)pyrene (BaP) on HepG2 cells by using a UHPLC/MS-TOF method. This rapid and simple method was developed for the identification, separation and quantification of 8-iPGF3 α , PGF3 α , 8-isoPGF2 α and 5-IPF2 α in cultured cells. The UHPLC/MS-TOF method was validated. The calculated limit of detection was in the range of 0.16–0.50 ng/mL, precision (% RSD): 1.2–2.1% and recoveries better than 88%. This method empowered qualitative and quantitative analysis of the selected individual prostaglandins derived from arachidonic acid and eicosapentaenoic acid from cell extracts.

Key words: human hepatocellular carcinoma cells HepG2, eicosapentaenoic acid, benzo(a)pyrene, isoprostanes, prostaglandins, UHPLC/MS-TOF method validation

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INTRODUCTION

Environmental and occupational exposure to chemicals may induce various diseases in individuals and populations leading to major public health problems. Polycyclic aromatic hydrocarbons (PAHs) are a large family of toxic compounds generated from the combustion of organic materials, diesel exhaust, and industrial waste. They are widely spread pollutants present in the atmosphere, water and soil.

Some PAHs are classified as human carcinogens, and also elicit a broad spectrum of toxic responses in humans and animals. Exposure to PAH results in a variety of molecular responses in an organism such as enzyme activation, oxidation, and/or signal transduction (Duan *et al.*, 2010; Hanzalowa *et al.*, 2010; Kang *et al.*, 2010; Pleil *et al.*, 2010; Teranishi *et al.*, 2010).

Many of these responses are mediated by the aryl hydrocarbon receptor (AhR)/aryl hydrocarbon nuclear translocator (ARNT) signalling pathway. AhR partici-

pates in NF- κ B signalling pathways regulating inflammation, immune responses, apoptosis, survival, and probably other functions. This aspect of biological roles of AhR is not clearly described and well known (Kung *et al.*, 2009; Puga *et al.*, 2009; Casado *et al.*, 2010; Quintana & Sherr 2013; Nguyen 2013).

A toxic potential of a chemical is related to its initial interaction with the target cell plasma membrane. Structural and chemical (lipid) composition of the membrane determines the overall rate of uptake of the xenobiotic from the extracellular space. The intramembranous retention of the compound directly influences the nature and rate of partitioning of the agent into intracellular space, where metabolic activation, detoxification, or direct reaction with macromolecules ensues. Binding of toxic substances, particularly those of lipophilic nature, to plasma membranes may result in perturbation of membrane-associated cell-regulatory mechanisms such as transport and activity of membrane-associated enzymes (Davidson *et al.*, 2012; Duan *et al.*, 2010; Eyster, 2007).

Apart from their carcinogenicity, PAHs may exert their negative effects on the human organism by induction of the oxidative stress. Oxidative stress resulting from an imbalance between prooxidants, including reactive oxygen species (ROS), and antioxidants in the organism may affect DNA, lipids, and proteins as well. Lipid peroxidation has two major impacts on the cell. It changes the properties of the cellular membranes, affects their structure and the activity of membrane-bound proteins, and it causes the formation of other reactive intermediates that propagate oxidative stress (Cadet *et al.*, 2010; Circu & Aw, 2010). Among the markers of lipid peroxidation, isoprostanes are considered to be the most reliable. They are formed by a free-radical attack on arachidonic acid localized in cellular membranes. Currently, 8-iso prostaglandin F2 α is the best characterized and the most often studied isoprostane (Brooks *et al.*, 2008; Guichardant & Lagerde, 2009; Milne *et al.*, 2006; Dahl & van Breemen, 2010).

The aim of this research was the evaluation of a rapid method for identification of lipid derivatives of eicosapentaenoic acid: prostaglandin F3 α (PGF3 α), 8-iso pros-

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Abbreviations: EPA, eicosapentaenoic acid; AA, arachidonic acid; PAHs, polycyclic aromatic hydrocarbons; BaP, benzo(a)pyrene; DMSO, dimethyl sulfoxide; FBS, fetal bovine serum; PBS, phosphate buffer saline; BHT, butylated hydroxytoluene; PGF3 α , Prostaglandin F3 α ; 8-iso PGF3 α , 8-iso Prostaglandin F3 α ; 5-IPF2 α -VI, 5-Isoprostane F2 α -VI; 8-isoPGF2 α , 8-iso Prostaglandin F2 α ; 15-F2t-Isoprostane; ACN, acetonitrile; MeOH, methanol; isoPs, isoprostanes

taglandin F3 α (8-iPGF3 α), and of arachidonic acid: 8-iso prostaglandin F2 α (8-iPGF2 α) and 5-isoprostane F2 α (5-iPF2 α) in HepG2 cultured cells, using ultrahigh performance liquid chromatography coupled to mass spectrometry with a time of flight mass analyzer (UHPLC/MS-TOF). Prostaglandin F2 α -d $_9$ (PGF2 α -d $_9$) and 8-iso prostaglandin F2 α -d $_4$ (8iPGF2 α -d $_4$) were used as the internal standards (ISs) throughout.

MATERIALS AND METHODS

Reagents. Prostaglandin F3 α (PGF3 α), 8-iso prostaglandin F3 α (8-iPGF3 α), 8-iso prostaglandin F2 α (8-iPGF2 α), 5-isoprostane F2 α (5-iPF2 α), prostaglandin F2 α -d $_9$ (PGF2 α -d $_9$) and 8-iso prostaglandin F2 α -d $_4$ (8iPGF2 α -d $_4$) were obtained from Cayman Chemical Company (Michigan, USA). The standards' concentration ranged between 0.31–5.0 ng/ml when using solutions of analytes (0.31, 0.63, 1.25, 1.5, 2.0, 2.5, 5.0). LC-MS grade methanol, ethanol, electrospray calibrant solution, butylated hydroxytoluene (BHT), eicosapentaenoic acid sodium salt and benzo(a)pyrene, potassium hydroxide, hydrogen peroxide, dimethyl sulfoxide (DMSO) were purchased from Sigma-Aldrich (St. Louis, USA). Analytical grade formic acid was supplied by Merck (Darmstadt, Germany). Water (18.2 M Ω cm, TOC < 5ppm) was ultra-purified and filtered through a Milli-Q Plus system (Millipore, Bedford, USA).

Standard solutions. IsoP stock solutions (5 μ g/mL) were prepared in ethanol with 0.1% formic acid and stored in a freezer (–20°C). Standard solutions were prepared daily by appropriately diluting stock solutions with methanol containing 5% formic acid.

Cultured cells. Human hepatocellular carcinoma cells used (American Type Cell Culture: Hep G2, HB-8065) were between 10th and 25th passages. Hep G2 cells were cultured in Eagle's Minimum Essential Medium supplemented with 10% FBS and 1% antibiotic solution (100 IU/mL penicillin, 0.1 mg/mL streptomycin) (ATTC; Gibco). Cells were maintained at 37°C in humidified atmosphere of 5% CO $_2$ in air and were finally seeded into a 6-well plate (Sarsted) at a density of 5 \times 10 5 cells/well in 2 mL of medium. At every step of the procedure, cell morphology was investigated by an inverted light microscope (Olympus). Cell viability during culturing was assessed with Trypan Blue Exclusion Test. HepG2 cells were treated with 4 μ L of eicosapentaenoic acid (EPA sodium salt dissolved in ethanol to the final concentration of 40 μ M/ μ L) for 24h combined with 1 μ L of benzo(a)pyrene dissolved in DMSO to the final concentration of 20 μ M/ μ L (for 2 h). Control cultures received the same concentration of ethanol (the final content did not exceed 0.12% v/v) and DMSO (the final content did not exceed 0.05% v/v) as experimental cells.

We used nontoxic concentrations for both EPA and BaP (effect on time-dose dependent study not presented). After incubation, the cells and media were collected. Trypsin–0.05 % EDTA solution was used to detach adherent cultures.

Cytotoxicity assay (MTT — 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide test). EPA was added to the cells at appropriate concentrations and incubated for 24 h, then 20 μ M BaP was added for 2 h. Cells cultured in the medium alone constituted the positive control (100% of growth); cells treated with 20 mM/l of hydrogen peroxide were employed as the negative control. The procedure was done as described above (Paško *et al.*, 2013).

Isolation of isoprostanes was done according to the method proposed by Milne *et al.*, 2006. Prior to extraction, 10 μ L of 0.01% BHT was added to each sample to prevent further oxidation. Then, PGF2 α -d $_9$ and 8iPGF2 α -d $_4$ (20 ng/ μ L) was added to samples as an internal standard. Solid phase extraction (SPE) was done using Bond Elute Plexa cartridges (Agilent Technologies). Next, SPE samples were reconstituted in 200 μ L of methanol containing 5% formic acid prior to UHPLC/MS-TOF analysis.

UHPLC/MS-TOF conditions. Identification and determination of isoprostanes 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 prostaglandins was carried out on a Synergi 4u Hydro-RP 80A column (150 \times 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 min — 20% to 22 min). The flow rate of the mobile phase was set to 0.2 ml/min. For each analysis, 10 μ L of sample solution was injected by an autosampler. Electrospray was used as an ion source. The optimized conditions were as follows: nebulizer pressure: 2.0 bar, dry gas: 7.0 l/min and temperature of dry gas: 220°C. The profile spectra were acquired in negative mode in the mass range of 50–1000 m/z with mass resolving power over 18000. Mass calibration was performed using a mixture of 10 mM sodium formate and isopropanol before each run. Data were processed by Data Analysis software (Bruker, Germany) and extracted ion chromatograms of all analytes were plotted using [M-H] $^-$ and calculated by IsotopicPattern software (Bruker, Germany).

RESULTS AND DISCUSSION

Development of UHPLC/MS-TOF method

At the beginning, to identify the studied IsoPs in low quantities the ESI-MS-TOF parameters were optimized using a single variable procedure for all 8 tested parameters of ESI source and mass spectrometer. Obtaining the highest abundance of m/z values corresponding to [M-H] $^-$ ions of the IsoPs provided a high sensitivity of the developed method. The best conditions of the tested

Table 1. Optimal mass spectrometry (ESI-MS-TOF) conditions

	Parameter	Optimal value
	End plate offset [V]	–500
	Capillary voltage [V]	+4800
ESI	Nebulizer pressure [Bar]	2.0
	Dry gas flow [l/min]	7.0
	Dry gas temperature [°C]	220
	Hexapole RF [Vpp]	600
MS-TOF	Transfer time [μ s]	30
	Pre pulse storage [μ s]	13

Table 2. Validation parameters of the UHPLC-MS-TOF method

IsoP	8-iPGF3 α	PGF3 α	8-iPGF2 α	5-iPF2 α	
LOD [ng/ml]	0.33	0.50	0.18	0.16	
LOQ [ng/ml]	1.02	1.62	0.61	0.38	
Linearity [ng/ml]	LOQ — 5.0				
Slope	0.0951	0.1553	0.0754	0.0367	
Intercept	-0.0515	-0.0936	0.0171	0.0907	
R ²	0.9947	0.9967	0.9986	0.9932	
% RSD (at 2.5 ng/ml), n=5	intraday	1.4	2.1	1.6	1.2
	interday	1.7	2.7	1.9	1.5
R % (at 2.5 ng/ml), n=4	90.0	88.5	91.2	89.3	
ME % (at 2.5 ng/ml), n=4	110	118	106	112	

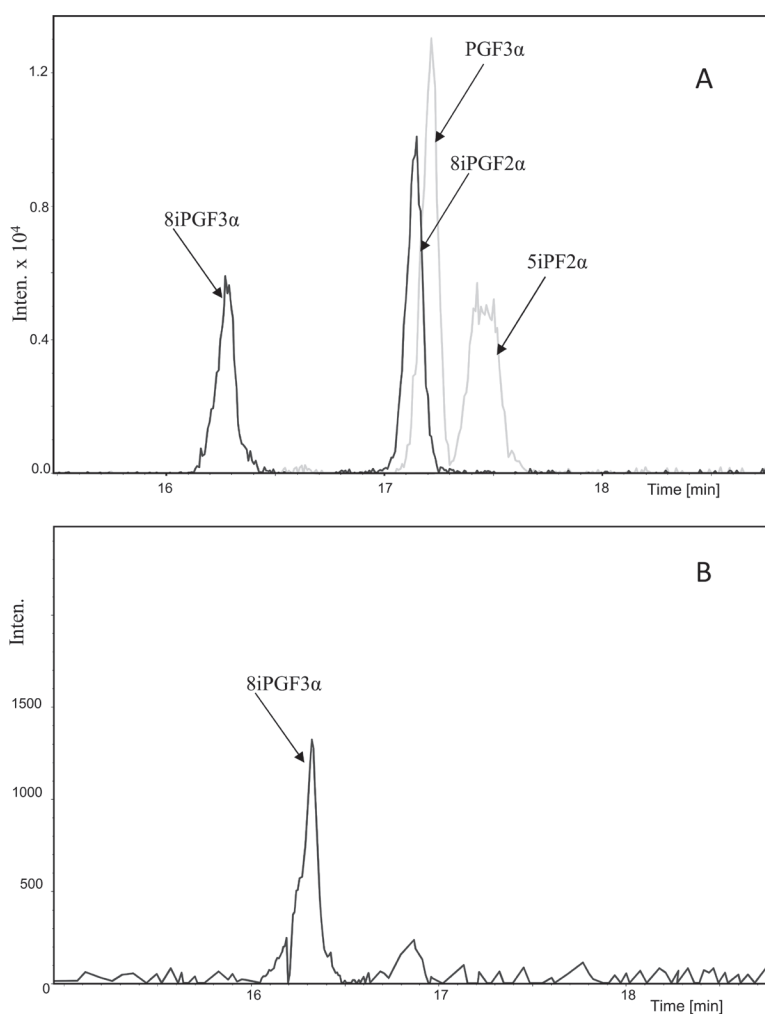


Figure 1 (a). Chromatogram of separation of the four studied IsoPs using UHPLC/MS-TOF method. (b) Chromatogram example of an experimental sample.

analytical system are shown in Table 1.

The UHPLC separation method was developed based on the chromatographic conditions published by Cao *et al.* (2008), Taylor *et al.* (2010) and Dahl *et al.* (2010). In order to separate the four isoprostanes analyzed (8-iPGF3 α , PGF3 α , 8-iPGF2 α , 5-iPF2 α) and the internal standards (PGF2 α -d9, 8iPGF2 α -d4), the mobile phase composition (ACN, MeOH, 0.01% formic acid and 0.01% acetic acid), flow rate (0.2, 0.3, 0.4 ml/min), column temperature (25, 30, 35, 40, 45, 50°C) and time of measurement (22, 24, 26, 32 min) were tested (for final, optimal condition see: Section UHPLC/MS-TOF conditions). The [M-H]⁻ masses with the defined retention time (Rt) for the studied IsoPs were: 8-iPGF3 α $m/z = 351.217 \pm 0.005$ (Rt = 16.3 min), PGF3 α $m/z = 351.217 \pm 0.005$ (Rt = 17.1 min), 8-iPGF2 α $m/z = 353.232 \pm 0.005$ (Rt = 17.2 min), 5-iPF2 α $m/z = 353.232 \pm 0.005$ (Rt = 17.4 min), PGF2 α -d9 $m/z = 362.289 \pm 0.005$ (Rt = 17.2 min), 8iPGF2 α -d4 $m/z = 357.257 \pm 0.005$ (Rt = 18.0 min). The chromatographic separation of the four IsoPs studied is depicted in Fig. 1.

Validation of the UHPLC/MS-TOF method

The optimized UHPLC/MS-TOF method was validated in terms of specificity, linearity, limits of detection and quantification, precision, recovery and matrix effects. The validation parameters were investigated at the concentration of 2.5 ng/ml, using standard solutions of the tested IsoPs. In order to define the recovery and matrix effects parameters, cell samples were analyzed. The calculated validation parameters are collected in Table 2.

Specificity. Specificity and selectivity of the method were assayed. To determine the effect of matrix components on the identification results, comparative analysis was carried out for a blank sample and the standard solutions as well as of the cell extracts. On the basis of the recorded chromatograms, no significant influence of other sample components on quantification was observed.

Linearity. Linearity of the method for each IsoP was tested in the concentration range 0.31–5.0 ng/ml

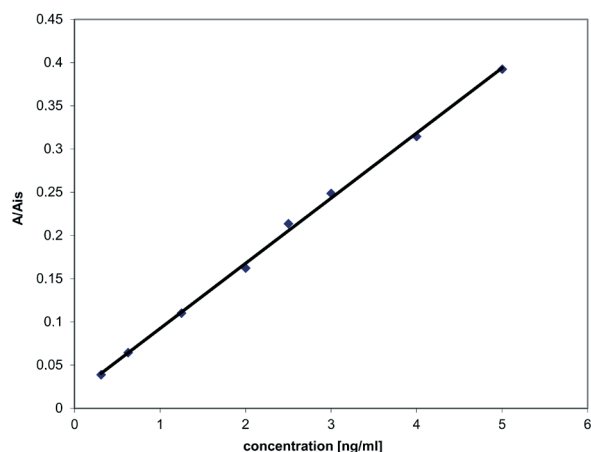


Figure 2. Calibration curve for 8-iPGF2 α .

using standard solutions of analytes (0.31, 0.63, 1.25, 1.5, 2.0, 2.5, 5.0). For each concentration of standard solution there were three injections. Calibration curves were calculated using peak-area ratios (drug/IS). PGF2 α -d9 was used as internal standard for 8-iPGF3 α and PGF3 α ; 8iPGF2 α -d4 was the internal standard for 8-iPGF2 α and 5-iPF α . The concentration of ISs was 20 ng/ml. Calibration curve for 8-iPGF2 α is presented at Fig. 2.

Limit of detection and limit of determination. Limits of detection (LOD) and determination (LOQ) were obtained experimentally, after the analysis of standard solutions of the studied isoprostanes. Limit of determination was calculated at the base of signal/noise as three and the limit of detection at the base signal/noise as ten.

Precision. Precision (% RSD) was investigated at the concentration level of 2.5 ng/ml. Precision of the UHPLC-MS-TOF method (% RSD) was calculated as reproducibility of the determined concentration and estimated by analysis of five separately prepared standard solutions of IsoP. The measurements were repeated on four different days. In this way, intraday and interday precision was evaluated.

Accuracy (Recovery). Accuracy of the method was defined in terms of isoprostanes recovery (R %). It was expressed by a ratio of the analytical signal obtained for IsoP which was added to a blank sample before extraction, to the analytical signal obtained for the same studied IsoP which was added after extraction in the same concentration (2.5 ng/ml, including enrichment factor), at $n = 4$, as the standard solution.

Matrix effects. Matrix effects (% ME) were assayed by the post-extraction addition approach. They were evaluated by comparing the mean peak area of the analyte spiked after extraction (into extracted blank sample) with the mean peak area of the same studied IsoP which was in a standard solution in the same concentration (2.5 ng/ml), expressed in percentage, at $n = 4$. The matrix effect values less than 100% represent ionization suppression and values below 100% reveal signal enhancements.

Application of the UHPLC/MS-TOF method for cell culture analysis

The developed and validated UHPLC/MS-TOF method was then used to identify and determine prostaglandins in HepG2. Qualitative analysis was carried out by comparing the retention time of each IsoP at the chromatogram with the retention time of the reference stan-

dard of IsoP and based on the ion mass value $[M-H]^-$ of the IsoP. Quantitative analysis was performed using the validation parameters described previously. The isoprostane content was calculated using a calibration curve based on the registered analytical signal (peak area of the IsoP/peak area of the IS). The application of the UHPLC/MS-TOF method enabled the identification and determination of prostaglandin (8-iPGF3 α) formed by EPA in human HepG2 cells (at the concentration 4.43 ng/ml) (Fig. 1b).

Due to the strong procancerogenic potential and reactivity of benzo(a)pyrene metabolites, as well as the susceptibility of polyunsaturated fatty acids to oxidizing, this study was undertaken. According to the available literature, a molecular mechanism of carcinogenesis of polycyclic aromatic hydrocarbons (PAHs) is not clearly understood. Knowledge on isoprostanes' metabolism and other active fatty acid products still remains incomplete. A mechanism of action wasn't also clarified for lipoxins, resolvins and other anti-inflammatory derivative polyunsaturated fatty acids. Therefore, studies that could contribute to explaining and revealing the pathomechanism of illnesses involving inflammation are advisable and necessary.

The results of our investigation have shown that supplementation with EPA for 24h and adding 20 μ M BaP for 2 h did not inhibit the growth and proliferation of tumor cell line Hep G2. In the control samples of Hep G2 and in the cells supplemented with EPA, isoprostanes were not found. In the cells supplemented with EPA with addition of BaP, active lipid derivatives were identified. We detected lipid derivatives from EPA -prostaglandin PGF3 α and isoprostane 8-iPGF3 α as well as from arachidonic acid (8-iPGF2 α and 5-iPF2 α -VI). These findings strongly suggest that benzo(a)pyrene acts via cyclooxygenase pathway (formation of PGF3 α) and propagates oxidative stress by lipid oxidation. Oxidative stress caused by lipid peroxidation through free radicals is believed to be one of the key factors underlying several acute and chronic diseases, which cause high morbidity and mortality, and oxidative stress has been implicated in the ageing process.

In the typical Western diet, n-6 polyunsaturated fatty acids (PUFAs) dominate, resulting in the release of pro-inflammatory arachidonic acid-derived metabolites. n-3 fatty acids might have a protective or even therapeutic effect. This may be attributable to their impact on mediators of inflammation. The dietary intake of fatty acids affects production of eicosanoids, which are potent immune mediators being mainly synthesized from eicosapentaenoic acid (EPA; C20:5 n-3) and arachidonic acid (AA; C20:4 n-6). Docosahexaenoic acid (DHA) and EPA are n-3 PUFAs mainly derived from fish oils that competitively inhibit n-6 PUFA arachidonic acid metabolism, thus reducing generation of the inflammatory leukotrienes and prostaglandins as much as the production of cytokines from inflammatory cells (Gao *et al.*, 2006; Kitz *et al.*, 2010; Serhan *et al.*, 2011).

EPA not only can replace arachidonic acid in phospholipid bilayers but is also a competitive inhibitor of cyclooxygenase, reducing the production of 2-series PGs and thromboxane, in addition to the 4-series leukotrienes. These studies suggest that the beneficial effects of n-3 fatty acids may be mediated, in part, by the anti-inflammatory effects of oxidized EPA (Connor *et al.*, 2007; Gao *et al.*, 2006; Song *et al.*, 2009; Sheran *et al.*, 2011).

While the prostaglandins are produced as a result of cyclooxygenase enzyme activity, isoprostanes are generally thought to form non-enzymatically by free radical-mediat-

ed peroxidation of arachidonic acid and other unsaturated fatty acids. Separate evidence has suggested that cyclooxygenase activity may also contribute to isoprostane production in selected tissues. Due to the potential role of isoprostanes in the pathogenesis of disease, their cellular signalling pathways and biological effects have been under investigation (Eyster, 2007; Davidson *et al.*, 2012).

Toxicity of aromatic hydrocarbons often involves cellular alternations associated with oxidative stress (Parrish *et al.*, 1998). Although many of PAHs have been shown to interfere with immune responses, the mechanisms underlying immunotoxicity of these compounds are not fully understood (Hwang *et al.*, 2007). Kelley *et al.* (1997) discovered that benzo(a)pyrene up-regulates cyclooxygenase-2 gene expression in oral epithelial cells. Dendele *et al.* (2013) observed protective action of n-3 fatty acids on benzo(a)pyrene-induced apoptosis. The authors showed that n-3 PUFA interferes with BaP-induced toxic effect through alternations of H⁺ homeostasis and inhibition of the N⁺/H⁺ exchanger (NHE-1) pathway.

Supplementation of fatty acids with EPA has been beneficial for cell viability, and it remains the subject of future studies. Study of eicosapentaenoic acid derivatives and their physiological role may provide valuable insights into the role of EPA in human physiology and pathophysiology. The identity and activity of key mediators is a crucial issue, and novel intermediates associated with prostanoid, cannabinoid, resolving and endoperoxide pathways are providing new therapeutic opportunities (Eyster, 2007; Sehran *et al.*, 2011; Davidson *et al.*, 2012).

In summary we demonstrate, for the first time, that eicosapentaenoic acid (EPA) has an anti-oxidative stress effect under benzo(a)pyrene exposition. Our findings strongly suggest that EPA plays a role in enhancement of the anti-oxidant defence and has a high therapeutic value, and thus should be one of the therapeutic approaches due to its dynamically modulatory properties.

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REFERENCES

Brooks JD, Milne GL, Yin H, Sanchez SC, Porter ND, Morrow JD (2008) Formation of highly reactive cyclopentenone isoprostane compounds (Δ^3/Δ^3 -Isoprostanes) *in vivo* from eicosapentaenoic acid. *J Biol Chem* **283**: 12043–12055.

Cadet J, Douki T, Ravanat J-L (2010) Oxidatively generated base damage to cellular DNA. *Free Radic Biol Med* **49**: 9–21.

Casado FL, Singh KP, Gasiewicz TA (2010) The aryl hydrocarbon receptor: Regulation of hematopoiesis and involvement in the progression of blood diseases. *Blood Cells Mol Dis* **44**: 199–206.

Cao H, Xiao L, Park GY, Wang X, Azim AC, Christman JW, van Breemen RB (2008) An improved LC-MS/MS method for the quantification of prostaglandins E2 and D2 production in biological fluids. *Anal Biochem* **372**: 41–51.

Circu ML, Aw TY (2010) Reactive oxygen species, cellular redox systems, and apoptosis. *Free Rad Biol Med* **48**: 749–762.

Connor KM, SanGiovanni JP, Lofqvist C, Aderman CM, Chen J, Higuuchi A, Hong S, Pravda EA, Majchrzak S, Carper D, Hellstrom A, Kang JX, Chew EY, Salem Jr N, Serhan CN, Smith LEH (2007) Increased dietary intake of n-3-polyunsaturated fatty acids reduces pathological retinal angiogenesis. *Nat Med* **13**: 868–873.

Dahl JH, van Breemen RB (2010) Rapid quantitative analysis of 8-isoprostaglandin-F2 α using liquid chromatography-tandem mass spec-

trometry and comparison with an enzyme immunoassay method. *Anal Biochem* **404**: 211–216.

Dendele B, Tekpli X, Hardonnere K, Holme JA, Debure L, Catheline D, Arlt VM, Nagy E, Phillips DH, Rvrebř S, Mollerup S, Poet M, Chevanne M, Rioux V, Dimanche-Boitrel M-T, Sergent O, Lagadic-Gossman D (2013) Protective action of n-3 fatty acids on benzo[a]pyrene-induced apoptosis through the plasma membrane remodeling-dependent NHE1 pathway. *Chem Biol Interact* <http://dx.doi.org/10.1016/j.cbi.2013.11.002> (in press).

Davidson J, Rotondo D, Rizzo MT, Leaver HA (2012) Therapeutic implications of disorders of cell death signalling: membranes, micro-environment, and eicosanoid and docosanoic metabolism. *B J Pharmacol* **166**: 1193–1210.

Duan H, Jiang Y, Zhang H, Wu Y (2010) MiR-320 and miR-494 affect cell cycles of primary murine bronchial epithelial cells exposed to benzo[a]pyrene. *Toxicol in Vitro* **24**: 928–935.

Eyster KM (2007) The membrane and lipids as integral participants in signal transduction: lipid signal transduction for the non-lipid biochemist. *Advan Physiol Educ* **31**: 5–16.

Gao L, Yin H, Milne GL, Porter NA, Morrow JD (2006) Formation of F-ring isoprostane-like compounds (F3-isoprostanes) *in vivo* from eicosapentaenoic acid. *J Biol Chem* **281**: 14092–14099.

Guichardant M, Lagarde M (2009) Analysis of biomarkers from lipid peroxidation: A comparative study. *Eur J Lipid Sci Technol* **111**: 75–82.

Hanzalova K, Rossner Jr P, Sram RJ (2010) Oxidative damage induced by carcinogenic polycyclic aromatic hydrocarbons and organic extracts from urban air particulate matter. *Mutation Res* **696**: 114–121.

Hwang J-A, Lee JA, Cheong S-W, Youn H-J, Park J-H (2007) Benzo(a)pyrene inhibits growth and functional differentiation of mouse bone marrow — derived dendritic cells, Downregulation of RelB and eIF3 p170b by benzo(a)pyrene. *Toxicol Lett* **169**: 82–90.

Kang Y, Cheung KC, Wong MH (2010) Polycyclic aromatic hydrocarbons (PAHs) in different indoor dusts and their potential cytotoxicity based on two human cell lines. *Environ Int* **36**: 542–547.

Kelley DJ, Mestre JR, Subbaramaiah K, Sacks PG, Schantz SP, Tanabe T, Inoue H, Ramonetti JT, Dannenberg AD (1997) Benzo[a]pyrene up-regulates cyclooxygenase-2 gene expression in oral epithelial cells. *Carcinogenesis* **18**: 795–799.

Kitz R, Rose MA, Schubert R, Beermann C, Kaufmann A, Böhles HJ, Schulze J, Zielen S (2010) Omega-3 polyunsaturated fatty acids and bronchial inflammation in grass pollen allergy after allergen challenge. *Respir Med* **104**: 1793–1798.

Kung T, Murphy KA, White LA (2009) The aryl hydrocarbon receptor (AhR) pathway as a regulatory pathway for cell adhesion and matrix metabolism. *Biochem Pharmacol* **77**: 536–546.

Milne GL, Sanchez SC, Musiek ES, Morrow JD (2006) Quantification of F2-isoprostanes as a biomarker of oxidative stress. *Nat Protocols* **2**: 221–226.

Nguyen NT, Hanich H, Nakahama T, Kishimoto T (2013) The roles of aryl hydrocarbon receptor in immune responses. *Int Immunol* **25**: 335–343.

Parrish AR, Alejandro NF, Bowes III RC, Ramos KS (1998) Cytotoxic response profiles of cultured renal epithelial and mesenchymal cells to selected aromatic hydrocarbons. *Toxicol in Vitro* **12**: 219–232.

Paško P, Bukowska-Strakova K, Gdula-Argasinska J, Tyszka-Czochara M (2013) Rutabaga (*Brassica napus* L. var. *napobrassica*) seeds, roots, and sprouts: a novel kind of food with antioxidant properties and proapoptotic potential in Hep G2 hepatoma cell line. *J Med Food* **16**: 749–759.

Pleil JD, Stiegel MA, Sibus JR, Tabucchi S, Ghio AJ, Madden MC (2010) Cumulative exposure assessment for trace-level polycyclic aromatic hydrocarbons (PAHs) using human blood and plasma analysis. *J Chromatogr B* **878**: 1753–1760.

Puga A, Ma C, Marlowe JL (2009) The aryl hydrocarbon receptor cross-talks with multiple signal transduction pathways. *Biochem Pharmacol* **77**: 713–722.

Schober W, Pusch G, Oeder S, Reindl H, Behrendt H, Buters JTM (2010) Metabolic activation of phenanthrene by human and mouse cytochromes P450 and pharmacokinetics in CYP1A2 knockout mice. *Chem Biol Interact* **183**: 57–66.

Serhan CN, Petasis NA (2011) Resolvins and protectins in inflammation resolution. *Chem Rev* **111**: 5922–5943.

Taylor AW, Traber MG (2010) Quantitation of plasma total 15-series F2-isoprostanes by sequential solid phase and liquid-liquid extraction. *Anal Biochem* **396**: 319–321.

Song WL, Paschos G, Fries S, Reilly MP, Yu Y, Rokach Y, Chang CT, Patel P, Lawson JA, FitzGerald GA (2009) Novel eicosapentaenoic acid-derived F3-isoprostanes as biomarkers of lipid peroxidation. *J Biol Chem* **284**: 23636–23643.

Teranishi M, Toyooka T, Ohura T, Masuda S, Ibuki Y (2010) Benzo[a]pyrene exposed to solar-simulated light inhibits apoptosis and augments carcinogenicity. *Chem Biol Interact* **185**: 4–11.

Quintana FJ, Sherr DH (2013) Aryl hydrocarbon receptor control of adaptive immunity. *Pharmacol Rev* **65**: 1148–1161.