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Research paper

Oxidized LDL triggers changes in oxidative stress and inflammatory biomarkers in human macrophages

Oscar J. Lara-Guzmán[a], Ángel Gil-Izquierdo[b], Sonia Medina[b], Edison Osorio[c], Rafael Álvarez-Quintero[c], Natalia Zuluaga[a], Camille Oger[d], Jean-Marie Galano[d], Thierry Durand[d], Katalina Muñoz-Durango[a,*]

[a] Vidarium, Nutrition, Health and Wellness Research Center, Nutresa Business Group. Calle 8 Sur No. 50-67, Medellín, Colombia
[b] Research Group on Quality, Safety and Bioactivity of Plant Foods, Department of Food Science and Technology, CEBAS (CSIC), P.O. Box 164, 30100 Campus University Espinardo, Murcia, Spain
[c] Grupo de Investigación en Sustancias Bioactivas, Facultad de Ciencias Farmacéuticas y Alimentarias, Universidad de Antioquia, Calle 70 No. 52-21, Medellín, Colombia
[d] Institut des Biomolécules Max Mousseron (IBMM), UMR 5247, CNRS, University of Montpellier, ENSCM, Faculty of Pharmacy, Montpellier, France

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ABSTRACT

Oxidized low-density lipoprotein (oxLDL) is a well-recognized proatherogenic particle that functions in atherosclerosis. In this study, we established conditions to generate human oxLDL, characterized according to the grade of lipid and protein oxidation, particle size and oxylipin content. The induction effect of the cellular proatherogenic response was assessed in foam cells by using an oxLDL-macrophage interaction model. Uptake of oxLDL, reactive oxygen species production and expression of oxLDL receptors (CD36, SR-A and LOX-1) were significantly increased in THP-1 macrophages. Analyses of 35 oxylipins revealed that isoprostanes (IsoP) and prostaglandins (PGs) derived from the oxidation of arachidonic, dihomo gamma-linolenic and eicosapentaenoic acids were strongly and significantly induced in macrophages stimulated with oxLDL. Importantly, the main metabolites responsible for the THP1-macrophage response to oxLDL exposure were the oxidative stress markers 5-*epi*-5-F_{2t}-IsoP, 15-E_{1t}-IsoP, 8-F_{3t}-IsoP and 15-keto-15-F_{2t}-IsoP as well as inflammatory markers PGDM, 17-*trans*-$PGF_{3\alpha}$, and 11β-$PGF_{2\alpha}$, all of which are reported here, for the first time, to function in the interaction of oxLDL with THP-1 macrophages. By contrast, a salvage pathway mediated by anti-inflammatory PGs (PGE_1 and 17-*trans*-$PGF_{3\alpha}$) was also identified, suggesting a response to oxLDL-induced injury. In conclusion, when THP-1 macrophages were treated with oxLDL, a specific induction of biomarkers related to oxidative stress and inflammation was triggered. This work contributes to our understanding of initial atherogenic events mediated by oxLDL-macrophage interactions and helps to generate new approaches for their modulation.

1. Introduction

Macrophage foam cells play an important role in atherosclerosis development. They induce reactive oxygen species (ROS) production, inflammatory responses and accumulation of lipids, which lead to fatty streak formation in the vascular wall [1]. Hypercholesterolemia is directly linked to key events in plaque progression, during which oxidative stress (OS) and inflammation are relevant [2]. An increase in circulating oxLDL is considered to be a primary contributor to the induction of foam cells in the arterial intima, where massive uptake of oxLDL by macrophages occurs via scavenger receptors (i.e., SR-A and CD36) and lectin-like oxLDL receptor-1 (LOX-1) [3,4]. OxLDL also activates SR/Toll-like receptor cooperative signaling pathways in macrophages, leading to the induction of pro-inflammatory downstream signaling cascades, ROS production and IL-1β maturation via NLRP3 inflammasomes [5,6]. In atherosclerosis-related inflammation,

Abbreviations used: AA, arachidonic acid; AAPH, 2,2′-Azobis (2-methylpropionamidine) dihydrochloride; BHT, butylated hydroxytoluene; BMI, body mass index; CVD, cardiovascular diseases; C-SMP, cell-surface marker proteins; DGLA, Dihomo-gamma-linolenic acid; DLS, dynamic light scattering; ETDA, ethylenediaminetetraacetic acid; EPA, eicosapentaenoic acid; ESI, electrospray ionization; FRAP, ferric reducing antioxidant power assay; IsoP, isoprostane; LDL, low-density lipoprotein; LDH, lactate dehydrogenase; LC, liquid chromatography; oxLDL, oxidized low-density lipoprotein; MDA, malondialdehyde; MFI, mean fluorescence intensity; MRM, multiple reaction monitoring; MS, mass spectrometry; OS, oxidative stress; PBS, phosphate-buffered saline; PMA, phorbol 12-myristate-13-acetate; PGs, Prostaglandins; PUFA, polyunsaturated fatty acids; REM, relative electrophoretic mobility; ROS, reactive oxygen species; SPE, solid phase extraction; SR, scavenger receptor; TBARS, thiobarbituric acid-reactive substances; TLR, toll-like receptor; Trolox, (±) − 6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid; TX, thromboxane; UHPLC, ultra-high performance liquid chromatography

* Corresponding author.
E-mail address: kmunoz@serviciosnutresa.com (K. Muñoz-Durango).

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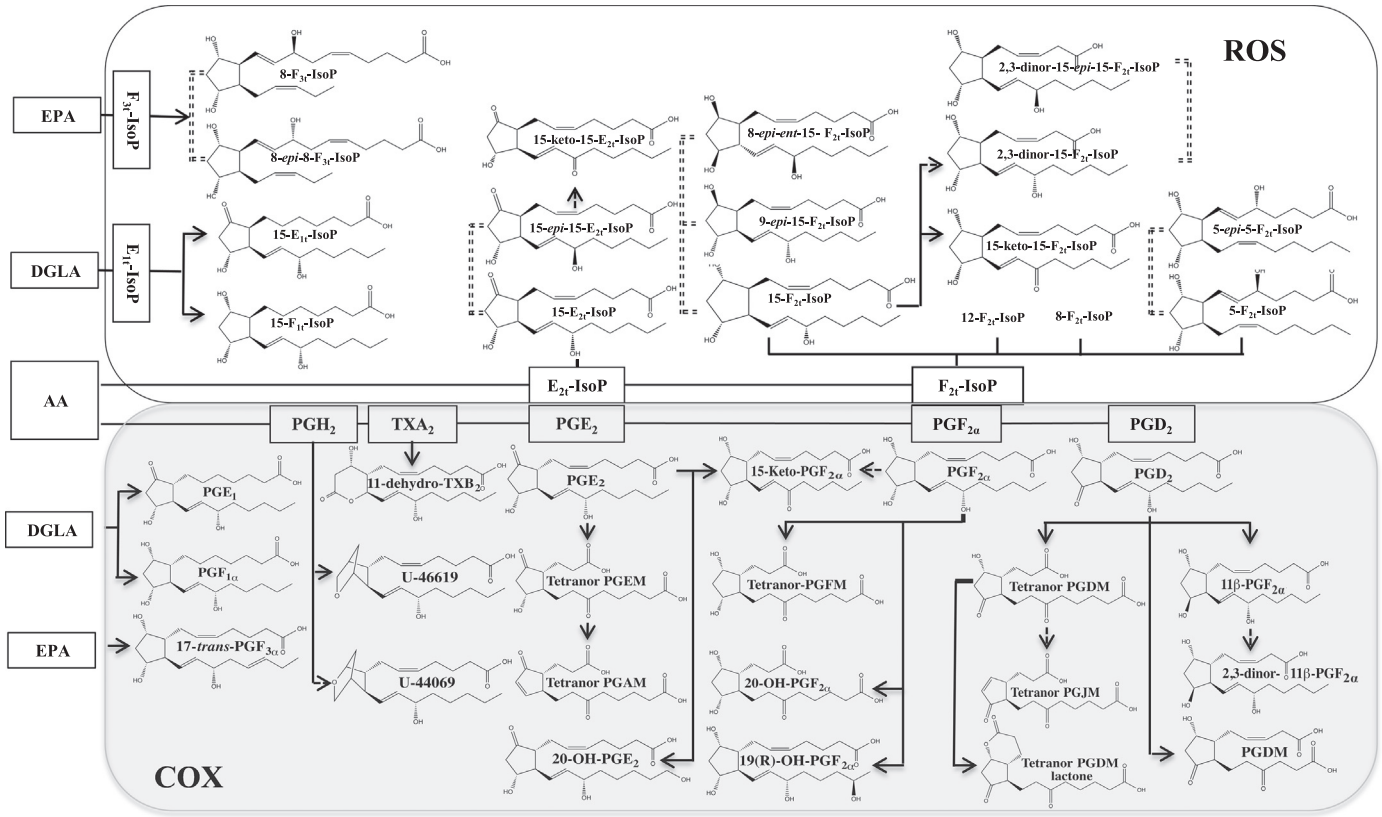

Scheme 1. Oxylipins derived from arachidonic acid C20:4 (AA), dihomo-gamma-linolenic acid C20:3 (DGLA) (omega-6 fatty acids) and eicosapentaenoic acid C20:5 (EPA) (omega-3 fatty acid). In gray: via cyclooxygenase (COX); in white: via reactive oxygen species (ROS). (= =) stereoisomers.

macrophages are regulated by numerous cytokines and chemokines, and the balance between pro- and anti-inflammatory signals determines plaque stability [1,5].

In the complex inflammatory context of atherosclerosis, macrophages also produce several lipid mediators known as oxylipins (isoprostanes and prostaglandins), which usually have negative effects [7]. Isoprostanes (IsoPs) are prostaglandin-like compounds that are produced *in vivo* by free radical-induced peroxidation of arachidonic acid (AA) [8]. They are generally considered to be OS markers and potentially mediate several of the adverse effects associated with oxidant injury [8]. These compounds are produced *in situ* in membrane phospholipids and are then released in their free form into circulation [9]. Prostaglandins (PGs), by contrast, are bioactive signaling molecules derived from cyclooxygenase (COX) and subsequent PG synthase activity on AA [7]. Several oxylipins are recognized as biomarkers of acute or chronic inflammation and are related to several pathologies, such as atherosclerosis, diabetes, ischemia-reperfusion, hypertension and obesity, as well as smoking [7,10,11]. Therefore, assessment of PGs and IsoPs in biological samples offers an opportunity to understand lipid metabolism, determine potential targets and characterize the relationship between oxidative stress and inflammation in atherogenesis [12,13].

In macrophage foam cells, a full physicochemical characterization of oxLDL is fundamental to understanding the biology of the cell. OxLDL may exist in multiple forms with different degrees of oxidation under physiological and *in vitro* conditions [14]. However, the previous differences in results obtained using this model can be primarily attributed to the heterogeneity in oxLDL preparation. Therefore, it is imperative to standardize conditions, such as the oxidants used, time, exposure, concentration and LDL source, for oxLDL generation and subsequent characterization [14]. Lipid and protein LDL modifications can be monitored by thiobarbituric acid-reactive substances (TBARS), relative electrophoretic mobility (REM) and particle size, which provide information concerning the features of oxLDL [15–17]. Related to oxylipin determination, the most suitable technique for simultaneous assessment of PGs and IsoPs in biological samples is UPLC/MS/MS [18]; through targeted lipidomics, hundreds of lipids in foam cells have been determined [18,19]. This analytical strategy has expanded the understanding of the effects of lipid mediators in the regulation of diverse cellular processes [20], as well as the role of oxylipins in macrophages and foam cells during the atherogenic process [19,21].

Despite the extensive literature concerning oxLDL-macrophage interactions and foam cell formation, both the induction effect of oxLDL on oxylipin production in macrophages as well as its association with biomarkers related to atherosclerotic lesions are poorly understood. To investigate the relationship between OS, inflammation and foam cell formation, we utilized a targeted lipidomic approach to perform functional analyses on THP-1 macrophages treated with human oxLDL. The aim of this study was to generate oxLDL under controlled conditions and to fully characterize and assess its effects on THP-1 macrophage oxylipin profiles. A total of 35 oxylipins related to inflammation and OS were screened by UHPLC–QqQ–MS/MS [22]. Remarkably, we report for the first time the induction of 8 PGs and 8 IsoPs as a result of oxLDL-THP-1 macrophage interactions, two of which have anti-inflammatory activity.

2. Material and methods

2.1. Chemicals and reagents

All LC–MS grade solvents, sodium acetate, trichloroacetic acid and $FeCl_3$ were obtained from J.T. Baker (Phillipsburg, New Jersey, USA). Formic acid was purchased from Panreac (Castellar Del Vallés, Barcelona, Spain). 2,4,6-Tris (2-pyridyl)-s-triazine (TPTZ), 2,2′-azobis (2-methylpropionamidine) dihydrochloride (AAPH), (±)−6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylicacid (Trolox), 2-

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