ΕΛΛΗΝΙΚΗ ΕΠΙΘΕΩΡΗΣΗ ΔΙΑΙΤΟΛΟΓΙΑΣ-ΔΙΑΤΡΟΦΗΣ 2011, 2(1), 11–18

Review

Bioactive lipids in atherosclerosis

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ABSTRACT Atherosclerosis is a low-grade systemic inflammatory condition and a dynamic process. Recent evidences suggest that anti-inflammatory products of polyunsaturated fatty acids such as lipoxins, resolvins, maresins and nitrolipids play a significant role in atherosclerosis by modulating the functions of platelets, leukocytes and macrophages and by protecting endothelial cells from the actions of reactive oxygen species. Hence, methods designed to enhance the formation of these bioactive anti-inflammatory lipids could be employed in the prevention and management of atherosclerosis.

Key-words: Atherosclerosis, polyunsaturated fatty acids, lipoxins, resolvins, protectins, free radicals, plaque, inflammation, nitrolipids.

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INTRODUCTION

Atherosclerosis, the major underlying cause for coronary heart disease (CHD), is a dynamic process. In majority of the instances, hyperlipidemia, diabetes mellitus, hypertension, obesity, hyperhomocysteinemia and smoking are the main risk factors for the development of atherosclerosis and CHD, conditions in which EFA (essential fatty acids) metabolism is abnormal such that plasma and tissue concentrations of γ-linolenic acid (GLA), dihomo-GLA (DGLA), arachidonic acid (AA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) in the phospholipid fraction are low.[1–8] Increased intake of polyunsaturated fatty acids (PUFAs especially in the form of GLA, DGLA, EPA and DHA) protects against the development of these diseases both in experimental animals[9–12] and humans,[13] though the exact mechanism of this protective action is unclear. GLA, DGLA, AA, EPA, and DHA form precursors to prostaglandin E_1 (PGE_1),

prostacyclin (PGI_2), PGI_3, lipoxins (LXs), resolvins, neuroprotectin D1 (NPD1), enhance NO generation, and interact with NO to form nitrolipids that have anti-inflammatory actions, prevent platelet aggregation, inhibit leukocyte activation and augment wound healing and resolve inflammation that may account for their beneficial actions. This implies that an altered EFA metabolism in the form of a block in the activity of Δ^6 and Δ^5 desaturases, which are essential for the formation of long-chain metabolites from dietary linoleic acid (LA, 18:2 ω-6) and α-linolenic acid (ALA, 18:3 ω-3), and inadequate formation of anti-inflammatory lipoxins, resolvins, protectins, maresins and nitrolipids from their precursor PUFAs could lead to the initiation, progression and aggravation of atherosclerosis.

LIPOXINS ARE POTENT ANTI-INFLAMMATORY MOLECULES

Lipoxins and their aspirin-triggered carbon-15 epimers are key mediators of endogenous anti-inflammation and resolution. Aspirin-triggered lipoxin A4 analog (ATL-1) have been shown to modulate reactive oxygen species (ROS) generation in endothelial cells. Pre-treatment of endothelial cells with ATL-1 completely blocked ROS production triggered by different agents, inhibited the phosphorylation and translocation of the cytosplamic NAD(P)H oxidase subunit p47 (phox) to the cell membrane as well as NAD(P)H oxidase activity and impaired the redox-sensitive activation of the transcriptional factor NF-κB, suggesting that lipoxins play a protective role against the development and progression of atherosclerosis and various cardiovascular diseases in which endothelial dysfunction is known to exist.[14] These results are supported in experiments performed with apolipoprotein E-deficient mice with (a) global leukocyte 12/15-lipoxygenase deficiency, (b) normal enzyme expression, or (c) macrophage-specific 12/15-lipoxygenase overexpression in which it was noted that 12/15-lipoxygenase expression protected mice against atherosclerosis via its role in the biosynthesis of lipoxin A4, resolvin D1, and protectin D1. These lipid mediators showed potent agonist actions on macrophages and vascular endothelial cells that reduced the magnitude of the local inflammatory response suggesting that a failure of local resolution mechanisms may underlie the unremitting inflammation that fuels atherosclerosis.[15] The evidence that lipoxins, resolvins and protectins are anti-inflammatory compounds and pro-inflammatory cytokines are elevated in atherosclerosis lends support to the belief that atherosclerosis is an inflammatory condition.

ATHEROSCLEROSIS IS A LOW-GRADE SYSTEMIC INFLAMMATORY CONDITION

An increase in the plasma concentrations of C-reactive protein (CRP), tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), myeloperoxidase (MPO), lipoprotein associated phospholipase A_2 (Lp-PLA_2), and lipid peroxides occurs in atherosclerosis suggesting that it is a low-grade systemic inflammatory condition.[16–24]

In atherosclerosis, circulating endothelial nitric oxide (eNO) levels are low,[25–27] reactive oxygen species (ROS) will be high, and anti-oxidant content will be low especially in the endothelial cells at atheroslcerosis-prone areas of the blood vessels.[28–32] The decrease in the production of eNO by endothelial cells may, in part, be due to enhanced levels of asymmetrical dimethylarginine (ADMA) that inhibits eNO generation that may lead to increased mortality due to cardiovascular diseases.[33–36] What is more significant is the observation that plasma ADMA concentrations were found to be positively related to internal carotid/bulb intimal-media thickness, suggesting that ADMA promotes subclinical atherosclerosis in a site-specific manner, with a greater proatherogenic influence at known vulnerable sites in the arterial tree.[37] In addition to ADMA, homocysteine also augments the formation of superoxide anion and reduces the synthesis and release of eNO.[38,39] Homocysteine markedly reduced the increase in haem oxygenase (HO) activity and HO-1 protein expression induced by sodium nitroprusside. High levels of homocysteine also abolished hypoxia-mediated HO-1 expression.[40]

It is noteworthy that NO reacts with PUFAs to yield their respective nitroalkene derivatives that can be detected in plasma. These nitroalkene derivatives, termed as nitrolipids, produce vascular relaxation, inhibit neutrophil degranulation and superoxide formation, and inhibit platelet activation.[41–43] Nitrolipids have endogenous PPAR-γ ligand activity and release NO.[43] These actions of nitrolipids prevent platelet aggregation, thrombus formation and atherosclerosis, and prevent inflammation.

Thus, PUFAs and their metabolites such as eicosanoids, lipoxins, resolvins, protectins, maresins and nitrolipids; various pro- and anti-inflammatory

cytokines, free radicals, nitric oxide (including ADMA) and various antioxidants seem to play critical role in the pathobiology of atherosclerosis.

PLATELETS, LEUKOCYTES AND ENDOTHELIAL CELLS IN ATHEROSCLEROSIS

The cross-talk among platelets, leukocytes and endothelial cells could determine the initiation and progression of atherosclerosis. For instance, under normal conditions, endothelial cells produce adequate amounts of PGE_1 from DGLA; PGI_2 from AA; LXs, resolvins, protectins and maresins from AA, EPA and DHA; formation of adequate amounts of nitrolipids such that the pro-inflammatory and pro-atherosclerotic events are successfully abrogated. Some of the pro-inflammatory and pro-atherogenic stimuli include: hemodynamic forces, hyperlipidemia, hypertension, hyperglycemia, smoking, etc; that induce the expression of pro-inflammatory genes, which initiate and accelerate atherosclerosis at the points of shear stress. These factors enhance infiltration of intima by leukocytes and macrophages, cause low-level activation of NF-κB and elevated expression of VCAM-1 and ICAM-1, IL-1, IL-6, MCP-1, as well as antioxidant genes glutathione peroxidase and glutathione-S- transferase 2, and pro-inflammatory eicosanoids such as TXA_2, PGE_2, $PGF_{2\alpha}$, LTs, and other PGs, TXs, and LTs, and increased production and release of free radicals and UCP (uncoupling proteins) expression occurs in endothelial cells, platelets, and leukocytes in atherosclerosis-susceptible regions, and endothelial cells themselves may show changes in cell shape and proliferation. These adverse events can be prevented and atherosclerosis process is arrested by the production of adequate amounts of PGE_1, PGI_2, PGI_3, LXs, resolvins, protectins, maresins, nitrolipids, NO, and anti-inflammatory cytokines such as IL-4, IL-10, TGF-β by endothelial cells. Thus, the balance between pro- and anti-inflammatory and pro and anti-atherosclerotic factors is tilted more towards pro-atherosclerotic and pro-inflammatory factors, atherosclerosis occurs.[44]

UNCOUPLING PROTEIN-1, ESSENTIAL FATTY ACIDS, AND ATHEROSCLEROSIS

The patchy manner in which atherosclerosis occurs suggests that arterial walls undergo regional disturbances of metabolism that include the uncoupling of respiration and oxidative phosphorylation, which may be characteristic of blood vessels being predisposed to the development of atheroslcerosis.[45] Oxidative stress is implicated in atherosclerosis. Mitochondrial electron transport accounts for most reactive oxygen species (ROS) production.[46] Uncoupling proteins (inner mitochondrial membrane anion transporters) allow protons to leak back into the mitochondrial matrix, thereby decreasing the potential energy available for ADP phosphorylation and ROS generation. Superoxide anion activates uncoupling proteins[47,48] that, in turn, limit further superoxide generation by dissipating proton motive force and thus, decreases oxidative stress. Uncoupling decreases glucose-induced ROS formation and abrogates pathways associated with vascular damage in endothelial cells *in vitro*.[49] In contrast, UCP-2 in macrophages decreases ROS and atheroslcerosis.[50] Although, these results appear to be in conflict with the proposal that inefficient vascular metabolism is detrimental, it is known that uncoupling agents produce smooth muscle contraction and cause hypertension,[51] and it was reported that respiratory uncoupling is increased in the aortae of experimental animals that are susceptible to atherosclerosis.[45] These results imply that the efficiency of vascular wall energy metabolism could be a determinant of atherosclerotic lesion development. UCP-1 expression in aortic smooth muscle cells causes hypertension and increases atheroslcerosis without affecting cholesterol levels.[46,52] This increase in UCP-1 expression also enhanced superoxide anion production and decreased the availability of NO, suggesting that oxidative stress has been elevated. This implies that inefficient metabolism in blood vessels causes atherosclerosis.

One of the earliest signs of atherosclerosis is the development of abnormal mitochondria in smooth muscle cells,[53] suggesting that mitochondrial dysfunction triggers the disease. Arteries have marginal oxygenation[54] and hypoxia reduces the respiratory control ratio.[55] Uncoupled respiration precedes atherosclerosis at lesion-prone sites but not at the sites that are resistant to atherosclerosis.[45] Disease-free aortae have abundant concentrations of the essential fatty acid-linoleate, whereas fatty streaks (an early stage of atherosclerosis) are deficient in EFAs.[52,56,57] EFA deficiency promotes respiratory uncoupling[58,59] and atherosclerosis.[1,60,61] Oxidative stress increases ROS generation and decreases NO formation and/or availability to be associated with

smooth muscle expression of UCP-1. These results emphasize that local disturbances of metabolism in the arterial wall are responsible for atherosclerosis and vascular disease.

PUFAs IN ATHEROSCLEROSIS

Atherosclerotic plaque rupture is known to be responsible for sudden coronary events. Felton et al[62] reported that the concentrations of all fatty acids were increased at the edge of disrupted plaques compared with the center, but as a proportion of total fatty acids, ω-6 were lower, suggesting that ω-6 fatty acids have a significant role in atherosclerosis. It is possible that there is a close interaction between ω-3 and ω-6 fatty acids, which could influence one's susceptibility or resistance to atherosclerosis. It is interesting to note that EPA/DHA readily get incorporated into the atheromatous plaque, and patients treated with fish oil had more thick fibrous caps and no signs of inflammation compared with plaques in patients in the control and sunflower oil groups. Furthermore, the number of macrophages in plaques from patients receiving fish oil was lower than in the other two groups, suggesting that atherosclerotic plaques readily incorporate ω-3 PUFAs from fish-oil supplementation, inducing changes that can enhance stability of atherosclerotic plaques.[63] In contrast, trans-fatty acids may render atheromatous plaques unstable, partly by displacing ω-3 fatty acids, interfering with ω-3 fatty acid metabolism and activating inflammatory responses and endothelial dysfunction.[64,65]

In this context, the interaction between ω-3 and ω-6 fatty acids is particularly significant. DGLA increases the conversion of EPA to PGI_3, AA augmented the conversion of EPA to PGI_3, EPA enhances the tissue levels of DGLA leading to increase in the formation of PGE_1, events that prevent atherosclerosis. In contrast, trans-fats interfere with the formation of DGLA, AA, EPA, and DHA and thus, prevent the formation of anti-atherosclerotic molecules:PGE1, PGI2, PGI3, lipoxins, resolvins, protectins, maresins and nitrolipids and at the same time may augment the formation and/or action of LTs, and TXs that promote atherosclerosis. The beneficial action of statins (HMG-CoA reductase inhibitors) and glitazones (PPARs agonists) seem to be mediated by EFAs and their metabolites such as LXs, resolvins, and protectins,[66–72] which are potent anti-inflammatory molecules.[1,73–75] On the other hand, cholesterol and saturated fatty acids similar to trans-fats block the activities of both Δ6 and Δ5 desaturases and inhibit the conversion of dietary LA and ALA to their respective long-chain metabolites and render cell membrane more rigid.[1] Increase in the consumption of trans-fats, cholesterol, and saturated fatty acids enhanced,[76–78] whereas consumption of ω-3 fatty acids decreased the levels of inflammatory markers.[79] These evidences suggest that ω-3 and ω-6 fatty acids, trans-fats, saturated fatty acids and cholesterol modulate inflammation and thus, influence the pathobiology of atherosclerosis, CAD and stroke.

ATHEROPROTECTIVE ACTIONS OF ω-3 AND ω-6 FATTY ACIDS

It is evident from the preceding discussion that both ω-3 and ω-6 PUFAs interact with each other to prevent atherosclerosis, CAD, CVD, and stroke, though ω-3 EPA and DHA seem to be having a more dominant role compared to ω-6 in this beneficial action. PUFAs display a multitude of actions (such as ability to lower plasma triglycerides, cholesterol and apolipoprotein B and alter hemostatic system; see table 1 also for the actions of PUFAs on lipid metabolism) to prevent atherosclerosis.

CONCLUSIONS

Based on the preceding discussion, it is clear that atherosclerosis is a low-grade inflammatory condition and PUFAs (especially ω-3 EPA and DHA) are useful in its prevention and management. PUFAs also inhibit ACE and HMG-CoA reductase activities and behave as endogenous ACE inhibitors. Statins similar to PUFAs and their products such as lipoxins, resolvins, protectins,

TABLE 1. Summary of effects of PUFAs on nuclear receptors involved in the regulation of lipogenesis.

Nuclear receptor	Effects on gene regulation	Expected changes		
		TG	HDL	LDL
PPAR-α	↑	↓↓	↑	↓
LXR	↓	↓↓	↓	↓
FXR	↑	↓↓	↑	↑
HNF-4α	↓	↓↓	↓	↔

FXR=Farnesol X receptor; HDL=High-density lipoprotein; HNF-4α=Hepatocyte nuclear factor-4α; LDL=Low-density lipoprotein, LXR=Liver X receptor; PPAR-α=Peroxisome proliferator-activated receptor; ↑=Increase; ↓=Decrease; ↔=Neutral effect

maresins and nitrolipids suppress the production of pro-inflammatory cytokines, modulate SREBP pathway and thus, inhibit atheroslcerosis both by lowering plasma triglycerides and cholesterol levels (see table 1), and modulating inflammatory events.

These evidences suggest that atherosclerosis can be prevented/arrested if endothelial cells are able to produce adequate amounts of various PUFAs such that they in turn lead to the formation of beneficial PGE1, PGI2, PGI3, LXs, resolvins, protectins, maresins and nitrolipids that are capable of suppressing inflammation, expression of various adhesion molecules on the surface of endothelial cells, and prevent leukocyte, monocyte and macrophage infiltration of endothelial cells (see figure 1).

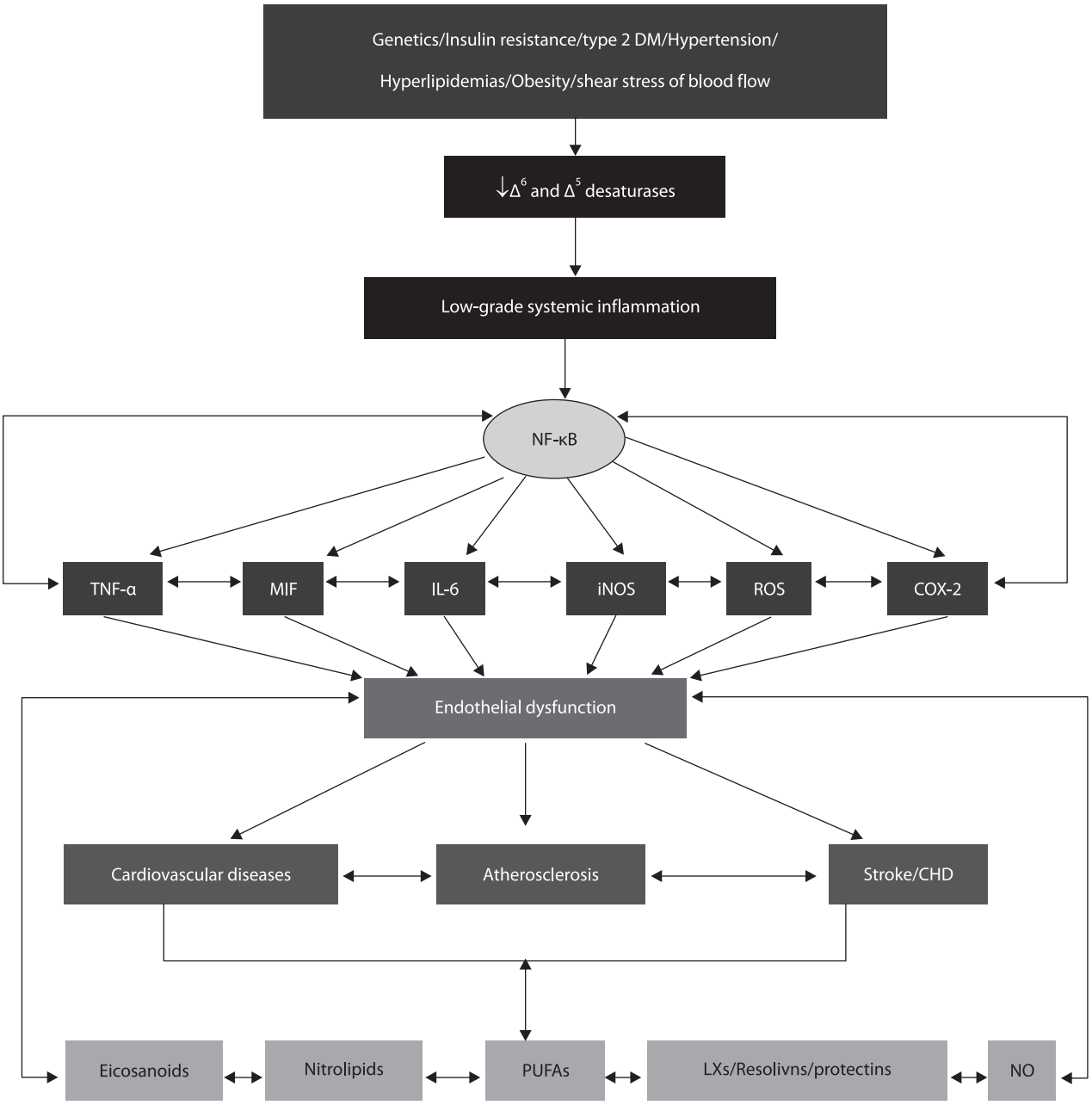

FIGURE 1. Scheme showing the relationship among various mediators of endothelial dysfunction and CHD/stroke and the role of PUFAs and their metabolites in these processes.

Βιοενεργά λιπίδια στην αθηροσκλήρωση

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ΠΕΡΙΛΗΨΗ Η αθηροσκλήρωση αποτελεί μια χαμηλού βαθμού συστηματική φλεγμονώδη κατάσταση και συγχρόνως μια δυναμική διαδικασία. Πρόσφατα δεδομένα υποστηρίζουν ότι τα αντιφλεγμονώδη παράγωγα των πολυακόρεστων λιπαρών οξέων, όπως οι λιποξίνες, οι ρεσολβίνες, οι μαρεσίνες και τα νιτρολιπίδια, διαδραματίζουν σημαντικό ρόλο στη διαδικασία της αθηροσκλήρωσης, τροποποιώντας τις λειτουργίες των αιμοπεταλίων, των λευκοκυττάρων και των μακροφάγων και προστατεύοντας τα ενδοθηλιακά κύτταρα από τις δράσεις των ενεργών μορφών οξυγόνου. Ως εκ τούτου, προσεγγίσεις που αποσκοπούν στην ενίσχυση του σχηματισμού αυτών των βιοενεργών αντιφλεγμονωδών λιπιδίων θα μπορούσαν να ενταχθούν στην πρόληψη και αντιμετώπιση της αθηροσκλήρωσης.

Λέξεις ευρετηρίου: Αθηροσκλήρωση, πολυακόρεστα λιπαρά οξέα, λιποξίνες, ρεσολβίνες, προτεκτίνες, ελεύθερες ρίζες, πλάκα, φλεγμονή, νιτρολιπίδια.

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