Oxidized lipids

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The addition of oxygen to lipids, in response to inflammatory and mitogenic stimuli, is an important process developed by biological systems to generate a wide spectrum of compounds both by enzymatic and non-enzymatic mechanisms. These oxidized lipids may serve as messengers for communication both within and between cells or may induce structural and metabolic changes in the cell. Since chronic inflammation has been proposed as an important risk factor for coronary events by making atherosclerotic plaques prone to rupture, extensive studies have been conducted to probe the involvement of the different pathways of lipid oxidation in the pathogenesis of coronary heart disease. The oxidation of arachidonic acid and 2-arachidonylglycerol through the activity of cyclooxygenase-2 may play a role in plaque instability through dysregulation of vascular tone and induction of endothelial dysfunction. Moreover, two families of biologically active mediators formed by free-radical catalyzed oxidation of arachidonic acid and phosphatidylcholine, i.e. isoprostanes and platelet activating factor-like lipids, respectively, may be involved. Clarification of the metabolic steps of lipid oxidation altered in unstable coronary artery disease will be of valuable help in identifying new cardiovascular markers for the prediction of the long-term risk of death from cardiac causes. The integration of this information with the presence of mutations in the genes encoding the enzymatic machinery of lipid oxidation will be useful in the selection of patients with increased risk of myocardial infarction.

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The addition of oxygen to lipids is an important process developed by biological systems to generate a wide spectrum of compounds both by enzymatic and non-enzymatic mechanisms. The oxidation of lipids precedes that of other biomolecules and unlike oxidized proteins, for example, which are destined for destruction, oxidized lipids may serve as messengers for communication both within and between cells or may induce structural and metabolic changes in the cell[1].

Both enzymatic and non-enzymatic lipid oxidation may be triggered by inflammatory and mitogenic stimuli. Thus, in the light of the involvement of chronic inflammation which, owing to the fact that it makes atherosclerotic plaques in coronary vessels prone to rupture[2], has been proposed as an important risk factor for coronary events, extensive studies have been conducted to probe the involvement of the different pathways of lipid oxidation in the pathogenesis of coronary heart disease. In this review, I will summarize the major findings and I will point out the possible future directions in studying the involvement of lipid oxidation in myocardial infarction. Further researches in this setting are necessary since half of the patients presenting with myocardial infarction do not have the classical risk factors[2].

Enzymatic lipid oxidation

Arachidonic acid, a polyunsaturated fatty acid containing 4 *cis* double bonds esterified at the *sn*-2 position of the glycerol backbone of membrane phospholipids, once released by the activity of phospholipase A_2, is oxidized in reactions catalyzed by the isoforms of prostaglandin (PG) H synthase (known as cyclooxygenase-1 and -2 or COX-1 and -2) or by the family of lipoxygenases that produces a broad range of biologically active compounds, i.e. prostanoids, leukotriene and lipoxins[3,4]. Lipoxygenases can peroxidize membrane lipids that remain esterified in membrane phospholipids with the formation of hydroxyeicosatetraenoic acids (HETEs) that, in turn, induce a series of functional cellular changes[4]. The enzyme 15-lipoxygenase has been implicated in the oxidation of low-density lipoproteins[5], a key event in the initiation of atherosclerosis[2].

The two isoforms of the enzyme COX catalyze the *bis*-dioxygenation of arachidonic acid, generating PGH_2, the precursor

of a family of biologically important and diverse prostanoids including PGE_2, prostacyclin (PGI_2) and thromboxane (TX) A_2 (Fig. 1A)[3]. COX-1 displays the characteristics of a "housekeeping gene" and is constitutively expressed throughout the body; it is involved in both human physiology (e.g. cytoprotection of the gastric mucosa and maintenance of the glomerular filtration rate and renal blood flow) and pathology (e.g. the enhanced platelet activation associated with cardiovascular risk factors)[6]. COX-1 is the only isoform expressed in platelets[7] and several lines of experimental and clinical evidence support the belief that prevention of myocardial infarction and ischemic stroke by aspirin is largely due to the permanent inactivation of platelet COX-1 and as a result to the synthesis of TXA_2[8], a potent agonist of platelet aggregation that has mitogenic and constrictive properties for vascular smooth muscle cells[9,10]. In contrast, COX-2 is the product of an "immediate-early gene" that is rapidly inducible and tightly regulated[11]. Under normal conditions, constitutive COX-2 expression is restricted to a limited number of tissues, such as the brain, kidney, reproductive system and vasculature; however, COX-2 is up-regulated in response to cytokines, bacterial endotoxins, growth factors and tumor promoters in a variety of cell types[12]. Thus, overexpression of COX-2 may act as an amplification loop in the propagation of the initial activation signal to adjacent cells. COX-2-dependent prostanoid biosynthesis may be involved in a variety of human pathological conditions, such as chronic arthritis, Alzheimer's disease, colorectal cancer and acute coronary syndromes. It has been proposed that COX-2 expression in nucleated cells (e.g. monocytes/macrophages and/or vascular cells) in response to an inflammatory *milieu* may be involved in the aspirin-insensitive TXA_2, biosynthesis detected in patients with acute coronary syndromes[13]. In fact, in unstable angina patients, the occurrence of episodes of enhanced biosynthesis *in vivo* of TXA_2, as reflected by the urinary excretion of its major enzymatic metabolites 11-dehydro-TXB_2 and 2,3-dinor-TXB_2, has been reported. This, despite the > 90% suppression of platelet COX-1 activity by low-dose aspirin, as monitored *ex-vivo*[14]. The involvement of COX-2 expression in this phenomenon can now be probed by the use of a new class of selective COX-2 inhibitors (coxibs) in this setting[6,15]. However, the interpretation of these studies may be hampered by the concomitant inhibition of the COX-2-dependent biosynthesis of PGI_2[16], a postulated atheroprotective mediator.

Cortisol secretion represents an endogenous mechanism which limits inflammation *in vivo*[17]. Similarly to synthetic glucocorticoids, cortisol has been shown to down-regulate COX-2 expression *in vivo* in experimental animals[18]. Studies aimed at ascertaining whether failure of the hypothalamic-pituitary-adrenal axis activation may be involved in enhanced TXA_2 biosynthesis, in response to inflammatory or mitogenic stimulation,

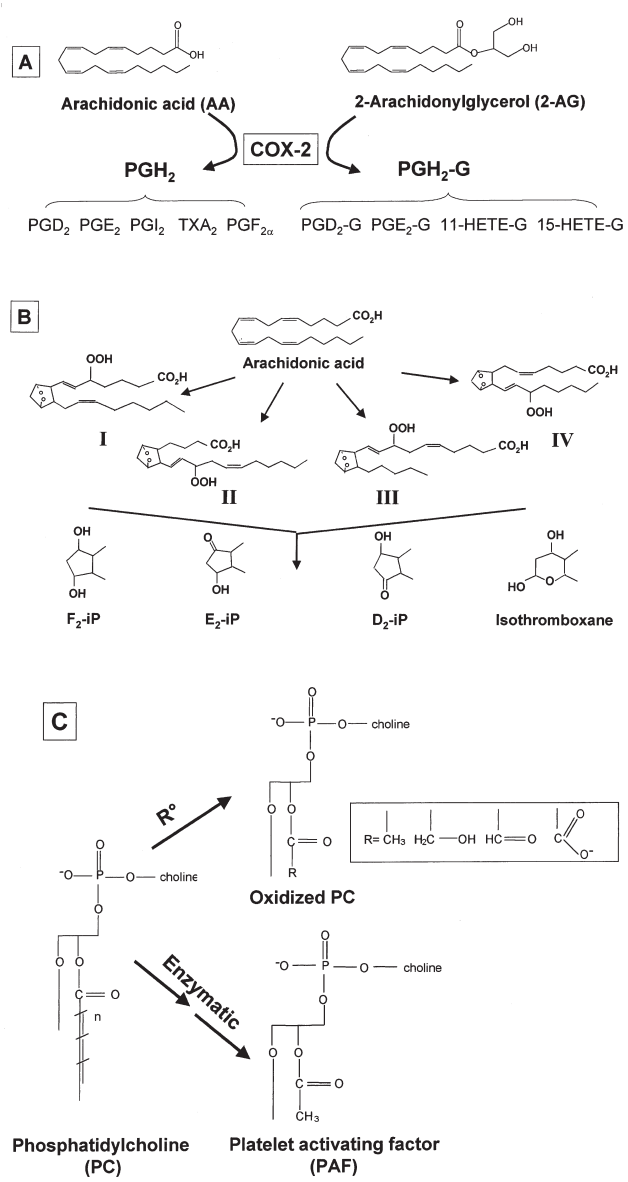

Figure 1. *Enzymatic and non-enzymatic pathways of lipid oxidation.* A: *oxygenation of arachidonic acid (AA) and 2-arachidonylglycerol (2-AG) to prostanoids [prostaglandin (PG) E_2, PGD_2, $PGF_{2\alpha}$, prostacyclin (PGI_2) and thromboxane (TX) A_2] and glyceryl-prostaglandins (PGE_2-G, PGD_2-G) and glyceryl-hydroxyeicosatetraenoic acids (11-HETE-G, 15-HETE-G), respectively, by cyclooxygenase (COX)-2.* B: *synthesis of isoprostanes (iP) and isothromboxanes by means of a free radical-catalyzed attack on esterified arachidonate. F_2-iPs possess a 1,3-dihydroxy-cyclopentane ring (PGF ring). Depending upon which of the labile hydrogen atoms is first abstracted by means of the free radical attack, up to 64 isomers in four structural classes can be generated.* C: *enzymatic synthesis of platelet activating factor (PAF) and oxidative fragmentation of alkyl phosphatidylcholine (PC). Some oxidatively fragmented alkyl PCs are PAF mimetic. From McIntyre et al.[41], modified.*

in acute coronary syndromes are currently underway. The results of these studies will provide valuable information on the possible mechanism of uncontrolled inflammation that has been proposed as a contributory factor to plaque instability[2].

The endogenous cannabinoid system appears to have vascular, neurological, immunological and reproductive functions[19]. Moreover, the cannabinoids exert

significant vascular effects in humans and laboratory animals. In particular, the cannabinoids cause vasodilation and hypotension[20]. The possible mechanisms of these effects include the inhibition of transmitter release from sympathetic nerve terminals, direct effects on vascular smooth muscle cells and effects on endothelial cell function. The identification of 2-arachidonylglycerol (2-AG) as an endogenous ligand for the central (CB1) and peripheral (CB2) cannabinoid receptors has prompted interest in enzymes capable of modifying or inactivating this endocannabinoid. It has been reported that 2-AG serves as a COX-2 selective substrate that is metabolized as effectively as arachidonic acid (Fig. 1A)[21]. The products of 2-AG oxygenation are PG and HETE esters that represent the first entries into a novel class of glyceryl eicosanoids. Thus, COX-2 may play a regulatory role in endocannabinoid signaling by decreasing 2-AG levels and reducing the tone at cannabinoid receptors. Whether this effect may contribute to plaque instability through the dysregulation of vascular tone and induction of endothelial dysfunction remains to be investigated.

Non-enzymatic lipid oxidation

Non-enzymatic lipid oxidation by free radicals is a detrimental event for the cell and organisms that employ numerous approaches to block the production of or limit the damage by these toxic agents. In fact, many oxidative chemical reactions may show exponential reaction rates, and some of the products are highly reactive species that modify proteins and DNA[1].

Isoprostanes (iPs) are a family of prostaglandin-like compounds formed non-enzymatically by means of a free radical-catalyzed attack on esterified arachidonate followed by enzymatic release from cellular or lipoprotein phospholipids[22-24]. F_2-iPs possess a 1,3-dihydroxycyclopentane ring (PGF ring). Depending upon which of the labile hydrogen atoms is first abstracted by free radical attack, up to 64 isomers in four structural classes can be generated (Fig. 1B). Compounds analogous to the F_2-iPs may be synthesized from other fatty acid substrates. Similarly, free radical-derived isomers of other PGs, leukotrienes, and epoxy-eicosatrienoic acids have been reported. iPs are so closely related to PGs that they exert their actions by capturing the receptors for PGs. F_2-iPs are detectable in human atherosclerotic lesions, particularly within monocyte/macrophages and smooth muscle cells[25]. They circulate in the plasma at low concentrations and are excreted in the urine[22-24]. 8-iso-$PGF_{2\alpha}$ (also referred to as $iPF_{2\alpha}$-III[26]) is an abundant F_2-iP formed *in vivo* in humans. This compound is of particular interest because it induces vasoconstriction and modulates the function of human platelets[23] through the interaction with TXA_2/PGH_2 receptors (TPs)[27]. However, unlike TXA_2, the interaction of 8-iso-$PGF_{2\alpha}$ with platelet TPs is associated with a morphologic change which does not result in the release of intracellular granules. This iP primes platelets to respond by aggregating fully in spite of the presence of subthreshold concentrations of other platelet agonists. 8-iso-$PGF_{2\alpha}$ has been proposed to act as a signal transduction mechanism of oxidative stress-dependent platelet activation. Presumably, many other iPs are biologically active; this will be revealed when the synthetic compounds will be available.

Altered generation of iPs has been reported in a variety of syndromes putatively associated with oxidative stress. These include coronary ischemia-reperfusion syndromes[28], Alzheimer's disease[29], adult respiratory distress syndrome, chronic obstructive pulmonary disease[30], and cystic fibrosis[31]. Enhanced synthesis of F_2-iPs has been reported in unstable angina[32] and in association with several cardiovascular risk factors, including hypercholesterolemia[33], diabetes mellitus[34], and cigarette smoking[35,36]. The latter conditions are characterized by increased lipid peroxidation in response to complex metabolic abnormalities or to various constituents of cigarette smoke. There is some evidence that iP formation may increase with age. Furthermore, even alcohol has been shown to increase iP synthesis[37].

The non-invasive measurement of F_2-iP may represent a useful tool for the identification of subjects with enhanced rates of lipid peroxidation who may benefit from antioxidant intervention. Moreover, the measurement of F_2-iP formation *in vivo* permits investigation of the dose-response relationship of antioxidants *in vivo*, thereby providing guidance for their rational use in relevant models of disease[38,39].

The same initial process (peroxidation of arachidonate esterified in phospholipids) can also yield another family of lipid mediators, the oxidized phosphatidylcholines with biologic activities similar to those of platelet activating factor (PAF) (Fig. 1C)[40,41]. They are potent biologically active compounds which trigger components of the immune and inflammatory systems. Some of these oxidation products activate cells expressing the PAF receptor. They activate human leukocytes, stimulate Ca^{2+} transients in platelets and the secretion of γ-interferon from human monocytes. These oxidatively fragmented PAF-like lipids also induce [^{3}H]thymidine incorporation into smooth muscle cells[41], an event relevant to the smooth muscle hypertrophy of atherosclerosis[2]. Unlike the synthesis of PAF that is tightly controlled by enzymes, its free radical-catalyzed oxidation products are potentially formed at high concentrations and may thus cause a pathological result. Due to the biological activity of these new oxidized lipids, further studies will be of valuable importance for the ascertainment of the role of these new products of non-enzymatic lipid oxidation in the development of cardiovascular diseases.

Conclusions

An extensive number of biologically active oxidized lipids can be formed in the cell both by enzymatic and non-enzymatic reactions. While enzyme-derived lipid formation may be controlled effectively by the use of selective drugs in humans, new, wide spectrum antioxidant drugs are probably required to efficiently block lipid oxidation in the cell. However, the development of a novel non-invasive analytical approach quantifying the antioxidant effect of conventional and new drugs *in vivo*, is provided by measurement of F_2-iP levels in plasma and urine.

The discovery of new families of oxidized lipids leads to the awareness that presumably we have uncovered only the surface of the complex network developed by the cell to modify lipids. Clarification of the metabolic steps of lipid oxidation altered in unstable coronary artery disease will be of valuable help for the identification of new cardiovascular markers which predict the long-term risk of death from cardiac causes. The integration of this information with knowledge regarding the presence of mutations in the genes encoding the enzymatic machinery of lipid oxidation will be useful for the selection of patients at an increased risk of myocardial infarction.

References

1. Prescott SM. A thematic series on oxidation of lipids as a source of messengers. J Biol Chem 1999; 274: 22901.
2. Ross R. Atherosclerosis - an inflammatory disease. N Engl J Med 1999; 340: 115-26.
3. Marnett LJ, Rowlinson SW, Goodwin DC, Kalgutkar AS, Lanzo CA. Arachidonic acid oxygenation by COX-1 and COX-2: mechanisms of catalysis and inhibition. J Biol Chem 1999; 274: 22903-6.
4. Brash AR. Lipoxygenases: occurrence, functions, catalysis, and acquisition of substrate. J Biol Chem 1999; 274: 23679-82.
5. Feinmark SJ, Cornicelli JA. Is there a role for 15-lipoxygenase in atherogenesis? Biochem Pharmacol 1997; 54: 953-9.
6. FitzGerald GA, Patrono C. The coxibs: rationally designed inhibitors of cyclooxygenase-2. N Engl J Med 2001, in press.
7. Patrignani P, Sciulli MG, Manarini S, Santini G, Cerletti C, Evangelista V. COX-2 is not involved in thromboxane biosynthesis by activated human platelets. J Physiol Pharmacol 1999; 50: 661-7.
8. Patrono C, Coller B, Dalen JE, et al. Platelet-active drugs: the relationships among dose, effectiveness, and side effects. Chest 2001; 119 (Suppl 1): S39-S63.
9. Hamberg M, Svensson J, Samuelsson B. Thromboxanes: a new group of biologically active compounds derived from prostaglandin endoperoxides. Proc Natl Acad Sci USA 1975; 72: 2994-8.
10. Dorn GW II. Role of thromboxane A_2 in mitogenesis of vascular smooth muscle cells. Agents Actions Suppl 1997; 48: 42-62.
11. Prescott SM. Is cyclooxygenase-2 the alpha and the omega in cancer? J Clin Invest 2000; 105: 1511-3.
12. Crofford LJ. COX-1 and COX-2 tissue expression: implications and predictions. J Rheumatol 1997; 24 (Suppl 49): 15-9.
13. Cipollone F, Patrignani P, Greco A, et al. Differential suppression of thromboxane biosynthesis by indobufen and aspirin in patients with unstable angina. Circulation 1997; 96: 1109-16.
14. Vejar M, Fragasso G, Hackett D, et al. Dissociation of platelet activation and spontaneous myocardial ischemia in unstable angina. Thromb Haemost 1990; 63: 163-8.
15. Hawkey CJ. COX-2 inhibitors. Lancet 1999; 353: 307-14.
16. McAdam BF, Catella-Lawson F, Mardini IA, Kapoor S, Lawson JA, FitzGerald GA. Systemic biosynthesis of prostacyclin by cyclooxygenase (COX)-2: the human pharmacology of a selective inhibitor of COX-2. Proc Natl Acad Sci USA 1999; 96: 272-7.
17. Schimmer BP, Paeker KL. Adrenocorticotropic hormone; adrenocortical steroids and their synthetic analogs; inhibitors and actions of adrenocortical hormones. In: Hardman JG, Limbird LE, Molinoff PB, Ruddon RW, Gilman AG, eds. Goodman and Gilman's. The pharmacological basis of therapeutics. New York, NY: McGraw-Hill, 1996: 1459-85.
18. Masferrer JL, Reddy ST, Zweifel BS, et al. In vivo glucocorticoids regulate cyclooxygenase-2 but not cyclooxygenase-1 in peritoneal macrophages. J Pharmacol Exp Ther 1994; 270: 1340-4.
19. O'Brein CP. Drug addiction and drug abuse. In: Hardman JG, Limbird LE, Molinoff PB, Ruddon RW, Gilman AG, eds. Goodman and Gilman's. The pharmacological basis of therapeutics. New York, NY: McGraw-Hill, 1996: 557-77.
20. Hillard CJ. Endocannabinoids and vascular function. J Pharmacol Exp Ther 2000; 294: 27-32.
21. Kozak KR, Rowlinson SW, Marnett LJ. Oxygenation of the endocannabinoid, 2-arachidonylglycerol, to glyceryl prostaglandins by cyclooxygenase-2. J Biol Chem 2000; 275: 33744-9.
22. Morrow JD, Hill KE, Burk RF, Nammour TM, Badr KF, Roberts LJ II. A series of prostaglandin F_2-like compounds are produced in vivo in humans by a non-cyclooxygenase free radical-catalyzed mechanism. Proc Natl Acad Sci USA 1990; 87: 9383-7.
23. Patrono C, FitzGerald GA. Isoprostanes: potential markers of oxidant stress in atherothrombotic disease. Arterioscler Thromb Vasc Biol 1997; 17: 2309-15.
24. Lawson JA, Rokach J, FitzGerald GA. Isoprostanes: formation, analysis and use as indices of lipid peroxidation in vivo. J Biol Chem 1999; 274: 24441-4.
25. Praticò D, Iuliano L, Mauriello A, et al. Localization of distinct F_2-isoprostanes in human atherosclerotic lesions. J Clin Invest 1997; 100: 2028-34.
26. Rokach J, Khanapure SP, Hwang SW, Adiyaman M, Lawson JA, FitzGerald GA. Nomenclature of isoprostanes: a proposal. Prostaglandins 1997; 54: 853-73.
27. Audoly LP, Rocca B, Fabre JE, et al. Cardiovascular responses to the isoprostanes $iPF_{2\alpha}$-III and iPE_2-III are mediated via the thromboxane A_2 receptor in vivo. Circulation 2000; 101: 2833-40.
28. Delanty N, Reilly M, Lawson JA, et al. 8-epi $PGF_{2\alpha}$ generation during coronary reperfusion. A potential quantitative marker of oxidant stress in vivo. Circulation 1997; 95: 2492-9.
29. Montine TJ, Markesbery WR, Morrow JD, Roberts LJ. Cerebrospinal fluid F_2-isoprostane levels are increased in Alzheimer's disease. Ann Neurol 1998; 44: 410-3.
30. Praticò D, Basili S, Vieri M, Cordova C, Violi F, FitzGerald GA. Chronic obstructive pulmonary disease is associated with an increase in urinary levels of isoprostane $F_{2\alpha}$-III, an index of oxidant stress. Am J Respir Crit Care Med 1998; 158: 1709-14.
31. Ciabattoni G, Davì G, Collura M, et al. In vivo lipid perox-

idation and platelet activation in cystic fibrosis. Am J Respir Crit Care Med 2000; 162: 1195-201.
32. Cipollone F, Ciabattoni G, Patrignani P, et al. Oxidant stress and aspirin-insensitive thromboxane biosynthesis in severe unstable angina. Circulation 2000; 102: 1007-13.
33. Davì G, Alessandrini P, Mezzetti A, et al. In vivo formation of 8-epi-prostaglandin $F_{2\alpha}$ is increased in hypercholesterolemia. Arterioscler Thromb Vasc Biol 1997; 17: 3230-5.
34. Davì G, Ciabattoni G, Consoli A, et al. In vivo formation of 8-iso-$PGF_{2\alpha}$ and platelet activation in diabetes mellitus. Effects of improved metabolic control and vitamin E supplementation. Circulation 1999; 99: 224-9.
35. Morrow JD, Frei B, Longmire AW, et al. Increase in circulating products of lipid peroxidation (F_2-isoprostanes) in smokers. Smoking as a cause of oxidative damage. N Engl J Med 1995; 332: 1198-203.
36. Reilly M, Delanty N, Lawson JA, FitzGerald GA. Modulation of oxidant stress in vivo in chronic cigarette smokers. Circulation 1996; 94: 19-25.
37. Aleynik SI, Leo MK, Lieber CS. Increased circulating products of lipid peroxidation in patients with alcoholic liver disease. Alcohol Clin Exp Res 1998; 22: 192-6.
38. Patrignani P, Panara MR, Tacconelli S, et al. Effects of vitamin E supplementation on F_2-isoprostane and thromboxane biosynthesis in healthy cigarette smokers. Circulation 2000; 102: 539-45.
39. Praticò D, Tangirala RK, Rader DJ, Rokach J, FitzGerald GA. Vitamin E suppresses isoprostane generation in vivo and reduces atherosclerosis in apoE-deficient mice. Nat Med 1998; 4: 1189-92.
40. Prescott SM, Zimmerman GA, McIntyre TM. Platelet-activating factor. J Biol Chem 1990; 265: 17381-4.
41. McIntyre TM, Zimmerman GA, Prescott SM. Biologically active oxidized phospholipids. J Biol Chem 1999; 274: 25189-92.