Biochimica et Biophysica Acta xxx (2013) xxx–xxx

Contents lists available at SciVerse ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbagen

Review

Biomarkers of lipid peroxidation in clinical material ☆

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ARTICLE INFO

Article history:
Received 2 February 2013
Received in revised form 14 March 2013
Accepted 17 March 2013
Available online xxxx

Keywords:
Biomarker
Hydroxycholesterol
Hydroxyeicosatetraenoic acid (HETE)
Hydroxyoctadecadienoic acid (HODE)
Isoprostane
Lipid peroxidation

ABSTRACT

Background: Free radical-mediated lipid peroxidation has been implicated in a number of human diseases. Diverse methods have been developed and applied to measure lipid peroxidation products as potential biomarkers to assess oxidative stress status in vivo, discover early indication of disease, diagnose progression of disease, and evaluate the effectiveness of drugs and antioxidants for treatment of disease and maintenance of health, respectively. However, standardized methods are not yet established.
Scope of review: Characteristics of various lipid peroxidation products as biomarkers are reviewed on the basis of mechanisms and dynamics of their formation and metabolism and also on the methods of measurement, with an emphasis on the advantages and limitations.
Major conclusions: Lipid hydroxides such as hydroxyoctadecadienoic acids (HODE), hydroxyeicosatetraenoic acids (HETE), and hydroxycholesterols may be recommended as reliable biomarkers. Notably, the four HODEs, 9-*cis*,*trans*, 9-*trans*,*trans*, 13-*cis*,*trans*, and 13-*trans*,*trans*-HODE, can be measured separately by LC–MS/MS and the *trans*,*trans*-forms are specific marker of free radical mediated lipid peroxidation. Further, isoprostanes and neuroprostanes are useful biomarker of lipid peroxidation. It is important to examine the distribution and temporal change of these biomarkers.
General significance: Despite the fact that lipid peroxidation products are non-specific biomarkers, they will enable to assess oxidative stress status, disease state, and effects of drugs and antioxidants. This article is part of a Special Issue entitled Current methods to study reactive oxygen species—Pros and cons

1. Introduction

Biomarkers are defined as measures that can be used as indicators of normal biological processes, pathological processes, or pharmacologic and/or biochemical responses to therapeutic/nutritional intervention. Biomarkers are used for health examination, diagnosis of pathologic processes, assessment of treatment response and prognosis, safe and efficient drug development, and evaluation of the effects of drugs, foods, beverages, and supplements. Lipid peroxidation is the major consequence of oxidative stress and cause of oxidative damage [1]. Various biomarkers for lipid peroxidation have been proposed, developed, and applied for biological samples from humans and experimental animals. Substantial evidence shows the association between the level of these biomarkers and development of many diseases. Accordingly, the lipid peroxidation products have received much attention as biomarkers of oxidative stress and diseases and also as indicators of antioxidant effects and the advantages and limitations of various biomarkers and methods of measurement have been the subject of extensive studies and arguments [1–10].

Lipids are vulnerable to oxidation by both enzymes and non-enzymatic oxidants. Especially, polyunsaturated fatty acids (PUFAs) possessing more than two *cis*-double bonds each separated by one methylene group and their esters are readily oxidized by free radical mediated chain oxidation, termed lipid peroxidation, which has been shown to impair membrane functions and inactivate proteins and enzymes, leading eventually to various disorders and diseases. Lipid peroxidation products have been accepted as toxic mediators, but now they are known to exert diverse biological effects [11–13].

The mechanisms and products of lipid peroxidation have been studied extensively and are now fairly well understood [1,14]. One of the characteristics of lipid peroxidation is that it proceeds by non-specific fashion. PUFAs and their esters having bis-allylic hydrogens are very reactive toward oxygen radicals and readily oxidized to produce lipid hydroperoxides as major primary products (Scheme 1). The major PUFA in vivo is linoleic (18:2), linolenic (18:3), arachidonic (20:4), eicosapentaenoic acid (20:5, EPA), and docosahexaenoic acid (22:6, DHA), which have one, two, three, four, and five bis-allylic methylene groups respectively. In contrast to lipoxygenase, peroxy radicals, chain carrying species of lipid peroxidation, attack these bis-allylic hydrogens non-selectively. For example, arachidonic acid has three bis-allylic

Abbreviations: CL, chemiluminescence; DHA, docosahexaenoic acid; DPPP, diphenylpyrenylphosphine; ELISA, enzyme linked immunosorbent assay; EPA, eicosapentaenoic acid; FL, fluorescence; GC, gas chromatography; H(P)ETE, hydro(pero)xyeicosatetraenoic acid; HPLC, high performance liquid chromatography; H(P)ODE, hydro(pero)xyoctadecadienoic acid; IsoP, isoprostane; MS, mass spectrometry; PC, phosphatidylcholine; PUFA, polyunsaturated fatty acid

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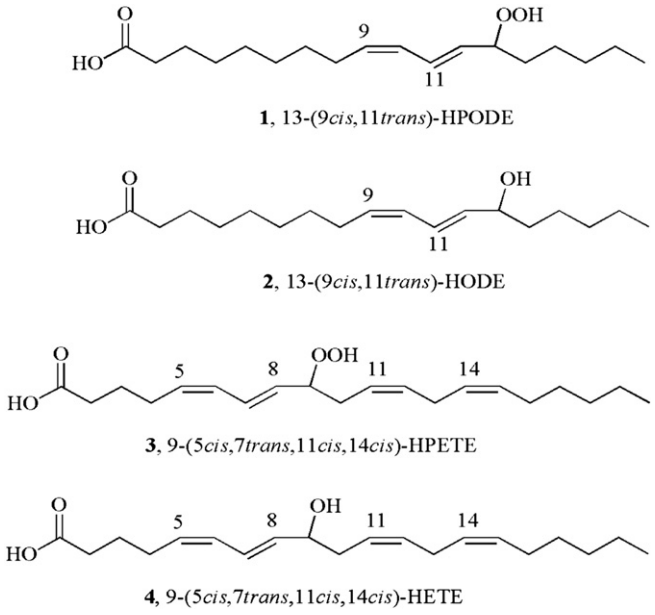
http://dx.doi.org/10.1016/j.bbagen.2013.03.020



Scheme 1. Chemical structure of hydroperoxyoctadecadienoic acid (HPODE, **1**), hydroxyoctadecadienoic acid (HODE, **2**), hydroperoxyeicosatetraenoic acid (HPETE, **3**), and hydroxyeicosatetraenoic acid (HETE, **4**).

methylene groups at carbon 7, 10, and 13 positions, which are equally attacked by peroxyl radicals and the resulting radicals may undergo several competing reactions to yield final products. Thus lipid peroxidation of PUFAs gives rise to diverse group of products with hundreds of regio-, stereo-, and enantio-isomers.

Cholesterol is also an important substrate of oxidation. It was accepted as an important component of cellular membranes and lipoproteins and also as a precursor to hormone synthesis, but it is oxidized in vivo substantially and its oxidation products play an important role as signaling messenger and biological mediators [15–18]. The oxidation products of cholesterol received attention as biomarkers of oxidative stress in vivo [19,20].

Lipids are oxidized not only by free radical pathways but also by non-radical oxidants and enzymes such as singlet oxygen, ozone, hypohalous acids, lipoxygenases, cyclooxygenases, and cytochrome P450. The same lipid oxidation products are formed by some of the free radical and non-radical oxidation. This point should be born in mind in the measurement and interpretation of data. It is important to understand the mechanisms of lipid oxidation by different oxidants in order to identify the responsible oxidant and assess the efficacy of antioxidant.

In the present article, the characteristics and measurement of lipid peroxidation products as a biomarker in clinical materials will be described, with special attention to the strengths and limitations as biomarkers on the basis of mechanisms and dynamics of their formation, metabolism, and excretion, rather than experimental details of protocols.

2. Lipid peroxidation products as biomarker: pros and cons

Various biomarkers of lipid peroxidation have been developed and applied to biological samples. Some representative biomarkers of lipid peroxidation are summarized in Table 1. The free radicals are too short-lived to be measured directly and stable products are measured. Early methods for determination of lipid peroxidation products depended largely on colorimetric assays involving the reaction of the lipid peroxidation products with chemical reagents that generated chromophores. For example, the iodometric assay is one of the oldest methods applied for measurement of lipid peroxidation products. In this method, iodine formed by the reaction of hydroperoxides and iodide is measured by titration [21]. This is a simple and convenient method, but due to a lack of specificity and sensitivity this method is not used today for clinical materials. With the advent of modern technologies such as chromatography, mass spectrometry, immunochemistry, and imaging technique, much more sophisticated methods are now available and applied for the identification, structure determination and quantification of lipid peroxidation products in biological samples. Nonetheless, there is still no method which is specific, accurate, sensitive, and quantitative enough to measure lipid peroxidation products in biological samples. The human samples contain low levels of lipid peroxidation products and their measurement may be interfered by other biological materials and also by artifactual oxidation during sampling, storage, and analysis. The characteristics of these biomarkers are described below.

Table 1
Biomarkers of lipid peroxidation [a].

Biomarker	Remarks/methods of measurement
Hydroperoxides	Primary product of lipid peroxidation, not stable/LC–UV, CL, FL, MS; DPPP
Hydroxides	Reduced form of hydroperoxides, HODE and HETE/LC–UV, MS; GC–MS; EIA
Isoprostanes	Free radical mediated oxidation product of arachidonic acid/LC–MS; GC–MS; EIA, RIA
Neuroprostanes	Free radical mediated oxidation product of DHA/LC–MS; GC–MS
TBARS, MDA	Thiobarbituric acid reactive substances measuring MDA and possibly others/spectrophotometry, HPLC
Conjugated diene	1,3-Diene of hydroperoxides and hydroxides/UV 234 nm
Ethane, pentane	Fragment product of hydroperoxides in exhaled gas/GC
Aldehydes, Ketones	Secondary products from hydroperoxides/LC; DNPH–UV/vis; EIA; RIA
LysoPC	Hydrolysis of PC by phospholipase A2/TLC, LC–MS/MS
7-Hydroxycholesterol	Reduction of 7-hydroperoxycholesterol, enzymatic oxidation/GC–MS
7- Ketocholesterol	Free radical oxidation of cholesterol/GC–MS
Oxidized LDL	Oxidatively modified LDL by multiple oxidants/EIA, RIA
LPO-modified proteins	Proteins modified by aldehydes/LC–MS; EIA; RIA
Lipofuscin	Fluorescence

[a] CL, chemiluminescence; DNPH, 2,4-dinitrophenylhydrazine; DPPP, diphenylpyrenylphosphine; EIA, enzyme immunoassay; FL, fluorescence; HETE, hydroxyeicosatetraenoic acid; HODE, hydroxyoctadecadienoic acid; GC, gas chromatography; LC, liquid chromatography; LPO, lipid peroxidation; Lyso PC, lysophosphatidylcholine; MS, mass spectrometry; RIA, radio immunoassay; TLC, thin layer chromatography; UV/V, ultraviolet/visible spectrophotometry.

2.1. Lipid hydroperoxides

Hydroperoxides are formed as the major primary product of lipid peroxidation of PUFA and cholesterol. Hydroperoxides are produced in the oxidation by singlet oxygen and lipoxygenase as well as free radical mediated lipid peroxidation [1]. Singlet oxygen oxidizes mono-olefins such as oleic acid and squalene as well as PUFA to give rise to hydroperoxides with concomitant migration of double bond. For example, the lipid peroxidation of linoleic acid by free radicals produces 9 and 13-hydroperoxyoctadecadienoic acid (HPODE), while the oxidation of linoleic acid by singlet oxygen gives four regio-isomers, 9, 10, 12, 13-HPODE, of which 10 and 12-HPODE do not contain conjugated diene. In contrast to lipoxygenases which attack specific position, peroxyl radicals attack bis-allylic hydrogens of PUFA equally; for example, peroxyl radicals abstract hydrogens at carbon 7, 10, and 13 positions of arachidonic acid at the same rate. The resulting carbon radicals react with molecular oxygen after rearrangement to yield thermochemically more stable 5, 8, 9, 11, 12, and 15-peroxyl radicals, which then abstract bis-allylic hydrogen to give the corresponding six regio-isomers of hydroperoxyeicosatetraenoic acid, HPETE. Since 8, 9, 11, and 12-peroxyl radicals, but not 5- and 15-peroxyl radicals, may also undergo intramolecular cyclization to give prostaglandin type

Please cite this article as: E. Niki, Biomarkers of lipid peroxidation in clinical material, Biochim. Biophys. Acta (2013), http://dx.doi.org/10.1016/j.bbagen.2013.03.020