Journal of Chromatography B, 1019 (2016) 202–209

Contents lists available at ScienceDirect

Journal of Chromatography B

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

Cholesterol as a natural probe for free radical-mediated lipid peroxidation in biological membranes and lipoproteins

Albert W. Girotti[a,*], Witold Korytowski[a,b]

[a] Department of Biochemistry, Medical College of Wisconsin, Milwaukee, WI, USA
[b] Department of Biophysics, Jagiellonian University, Krakow, Poland

ARTICLE INFO

Article history:
Received 30 July 2015
Received in revised form
17 December 2015
Accepted 18 December 2015
Available online 28 December 2015

Keywords:
Cholesterol
Cholesterol oxides
Free radicals
Lipid peroxidation
Thin layer chromatography
Phosphorimaging

ABSTRACT

We describe a relatively convenient and reliable procedure for assessing the magnitude of free radical-mediated (chain) lipid peroxidation in biological systems. The approach is based on use of radiolabeled cholesterol ([^{14}C]Ch) as a probe and determination of well-resolved oxidation intermediates/products ([^{14}C]ChOX species), using high performance thin layer chromatography with phorphorimaging detection (HPTLC-PI). In a lipid hydroperoxide-primed liposomal test system treated with ascorbate and a lipophilic iron chelate, the following well-resolved [^{14}C]ChOX are detected and quantified: 7α/7β-OOH, 7α/7β-OH, and 5,6-epoxide, their levels increasing with incubation time at 37 °C. [^{14}C]Ch also serves as an excellent probe for lipid peroxidation in lipoproteins and plasma membranes of mammalian cells. Because this approach utilizes Ch as a natural in situ probe, it eliminates potential artifacts associated with artificial probes such as spin traps and fluorophores.

© 2015 Elsevier B.V. All rights reserved.

1. Introduction

Oxidative stress-induced peroxidation of unsaturated phospholipids and cholesterol in cell membranes and lipoproteins is of considerable biomedical interest because of its possible involvement in pathophysiological conditions such as chronic inflammation, ischemia-reperfusion injury, neurodegeneration, and atherogenesis [1–4]. Oxidative stress arising when pro-oxidant pressure exceeds antioxidant capacity is accompanied by formation of reactive oxygen species (ROS) such as superoxide ($O_2^{-\bullet}$), hydrogen peroxide (H_2O_2), and hydroxyl radical ($HO^\bullet$), the latter being a strong indiscriminate oxidant that can initiate non-enzymatic free radical-mediated lipid peroxidation [3]. The initiating step might be abstraction of an allylic hydrogen from an unsaturated *sn*-2 fatty acyl group on a membrane phospholipid, e.g. 1 palmitoyl-2-linoleoyl-*sn*-glycero-phosphocholine (PLPC); see Eq. (1),

$$LH + HO^\bullet \rightarrow L^\bullet + H_2O \quad (1)$$

$$L^\bullet + O_2 \rightarrow LOO^\bullet \quad (2)$$

$$LOO^\bullet + L'H \rightarrow LOOH + L'^\bullet \quad (3)$$

$$LOOH + Fe^{2+} + O_2 \rightarrow OLOO^\bullet + OH^- + Fe^{3+} \quad (4)$$

$$OLOO^\bullet + L''H \rightarrow OLOOH + L''^\bullet \quad (5)$$

$$OLOOH + Fe^{2+} \rightarrow OLO^\bullet + OH^- + Fe^{3+} \quad (6)$$

$$OLO^\bullet + L'''H \rightarrow OLOH + L'''^\bullet \quad (7)$$

where LH denotes an unsaturated lipid. The resulting alkyl radical ($L^\bullet$) reacts rapidly with O_2 to give a peroxyl radical ($LOO^\bullet$) (Eq. (2)), which in turn can abstract an allylic hydrogen from another unsaturated lipid (L′H), giving a lipid hydroperoxide (LOOH) (Eq. (3)). The accompanying $L'^\bullet$ then begins the propagative phase of chain peroxidation (Eqs. (2) and (3)). Meanwhile, if suitably ligated

Abbreviations: AH^-, ascorbate; ALA, 5-aminolevulinic acid; Ch, cholesterol [(3β)-cholest-5-en-3-ol)]; ChOOH, cholesterol hydroperoxide; ChOX, cholesterol oxide(s); DFO, desferrioxamine; DMPC, 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine; $Fe(HQ)_3$, ferric 8-hydroxyquinoline; GPx4, type-4 glutathione peroxidase; HPLC-EC(Hg), high-performance liquid chromatography with mercury cathode electrochemical detection; HPTLC-PI, high-performance thin layer chromatography with phosphorimaging detection; LDL, low density lipoprotein; LH, unsaturated lipid; LOOH, lipid hydroperxide; PLOOH, phospholipid hydroperoxide; POPC, 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine; SPNO, spermine NONOate; SUV/LUV, small and large unilamellar vesicles (liposomes); 5α-OOH, 3β-hydroxy-5α-cholest-6-ene-5-hydroperoxide; 5α-OH, 5α-cholest-6-ene-3β,5-diol; 5,6>O, cholestan-5,6-epoxy-3β-ol; 6α/6β-OOH, 3β-hydroxycholest-4-ene-6α- and 6β-hydroperoxide; 7α/7β-OOH, 3β-hydroxycholest-5-ene-7α- and 7β-hydroperoxide; 7α/7β-OH, cholest-5-ene-3β,7α- and 7β-diol; 7=O, 3β-hydroxycholest-5-ene-7-one.

* Corresponding author at: Department of Biochemistry, Medical College of Wisconsin, Milwaukee, WI 53226, USA.
E-mail address: agirotti@mcw.edu (A.W. Girotti).

http://dx.doi.org/10.1016/j.jchromb.2015.12.034
1570-0232/© 2015 Elsevier B.V. All rights reserved.

iron (or other redox metal ion) and a reductant are available, LOOH can undergo one-electron reduction to an oxyl radical (LO•), which rearranges and reacts with O_2 to give an epoxyallylic peroxyl radical (OLOO•) (Eq. (4)) [5]. The latter in turn can induce a new round of chain peroxidation via hydrogen abstraction from another proximal lipid (Eq. (5)). The resulting hydroperoxide may also undergo iron-catalyzed one-electron reduction (Eq. (6)), triggering a new chain via OLO•, which is reduced to an alcohol (Eq. (7)). LOOHs can also arise in non-radical fashion, a key example being "ene" addition of singlet molecular oxygen ($^1O_2$), a photochemically-generated ROS, to an unsaturated lipid [4]. However, subsequent one-electron turnover of $^1O_2$-derived LOOHs can induce chain peroxidation reactions (Eqs. (4)–(7)) similar to those initiated by HO• [2–4].

Non-esterified cholesterol (Ch) is found in the outer layer of lipoproteins and in membranes of eukaryotic cells, most of it residing in the plasma membrane, where it comprises ~45 mol% of total lipid. As a monounsaturated lipid, Ch is susceptible to oxidative degradation, but less so than polyunsaturated fatty acyl groups in phospholipids. Oxidation occurring in the ring portion of Ch is predominantly non-enzymatic (free radical-mediated), whereas that occurring in the side-chain is usually enzymatic [6]. Free radical-mediated oxidation gives rise to a discrete number of cholesterol oxides (oxysterols), including hydroperoxides (ChOOHs), diols, epoxides, and ketone [6–8]. A lipid peroxyl radical can attack at the Ch double bond, giving epimeric 5,6-epoxides (5,6 > O) (Eq. (8)).

$$\mathrm{Ch} + \mathrm{OLOO}^{\bullet} \rightarrow 5,6 > \mathrm{O} + \mathrm{OLO}^{\bullet} \quad (8)$$

$$\mathrm{Ch} + \mathrm{OLOO}^{\bullet} \rightarrow 7^{\bullet} + \mathrm{OLOOH} \quad (9)$$

$$7^{\bullet} + O_2 \rightarrow 7 - \mathrm{OO}^{\bullet} \quad (10)$$

$$7 - \mathrm{OO}^{\bullet} + \mathrm{LH} \rightarrow 7 - \mathrm{OOH} + \mathrm{L}^{\bullet} \quad (11)$$

$$7 - \mathrm{OOH} + \mathrm{Fe}^{2+} \rightarrow 7 - \mathrm{O}^{\bullet} + \mathrm{OH}^{-} + \mathrm{Fe}^{3+} \quad (12)$$

$$7 - \mathrm{O}^{\bullet} + \mathrm{L'H} \rightarrow 7 - \mathrm{OH} + \mathrm{L'}^{\bullet} \quad (13)$$

$$7 - \mathrm{O}^{\bullet} + \mathrm{OLOO}^{\bullet} \rightarrow 7 = \mathrm{O} + \mathrm{OLOOH} \quad (14)$$

Alternatively, abstraction of a C-7 allylic hydrogen by a strong oxidant such as OLOO•, followed by O_2 addition and hydrogen abstraction from another lipid gives the 7α- and 7β-hydroperoxides (7—OOHs) (Eqs. (9)–(11)). As with LOOHs in general, iron-catalyzed one-electron reduction of either 7—OOH gives the corresponding diol (7—OH) (Eqs. (12) and (13)). Alternatively, the oxyl radical intermediate may undergo β-hydrogen scission in the presence of a peroxyl radical, giving the 7-ketone (7=O) (Eq. (14)). Structures of the Ch oxides (ChOX) referred to in Eqs. (8)–(14), and which are prominent in the analytical approach we describe, are shown in Fig. 1. It is important to note that whereas 7α- and 7β-OOH derive from free radical reactions, 5α-OOH and 6α/6β-OOH are generated exclusively by $^1O_2$ attack on Ch and can serve as unambiguous reporters of $^1O_2$ intermediacy in membrane oxidations [4,7,9].

Ongoing interest in free radical-mediated lipid peroxidation has stimulated the development of highly sensitive and specific techniques for detecting and quantifying this process. These techniques typically involve measurement of reactive intermediates such as hydroperoxides or end-products such as hydroxides and aldehydes. They range from relatively simple "bulk-type" methods, e.g. the classic thiobarbituric acid and iodometric assays [9] to more sophisticated methods involving high performance liquid or gas chromatography with high sensitivity/specificity post-column detection of LOOHs and other characteristic species [10–12]. Perceiving the need for more convenience and less complexity in monitoring chain lipid peroxidation, the authors introduced a novel approach based on use of radiolabeled Ch as a natural probe for free radical lipid peroxidation in biological membranes and lipoproteins [13]. In this approach, [^{14}C]Ch acts as a "sensor" of free

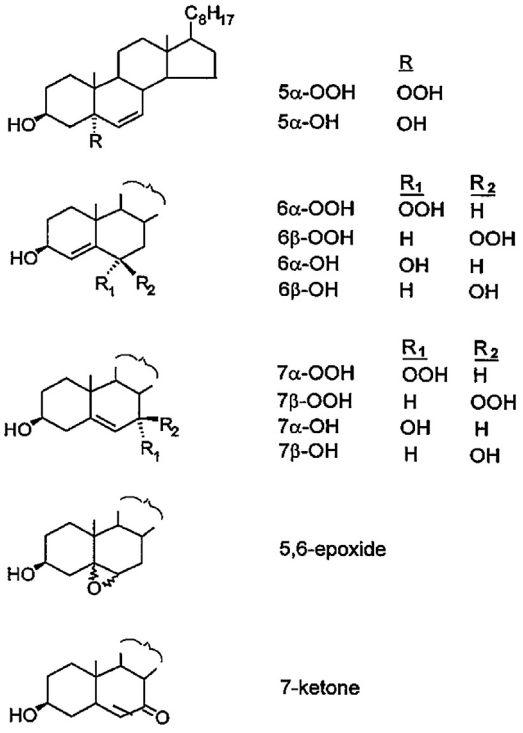

Fig. 1. Structures of cholesterol hydroperoxides and other oxides referred to in this article. Formal names of these species are provided in the list of abbreviations.

radical activity in its surroundings. The intermediates and products of [^{14}C]Ch oxidation, ([^{14}C]ChOX species) serve as reporters of damaging peroxidation and are detected by high-performance thin layer chromatography with phosphorimaging (HPTLC-PI). We describe several different protocols that illustrate how this unique approach can be used to detect/quantify chain peroxidation in a variety of test systems ranging from relatively simple liposomes to mammalian cells. A simplified procedural flow diagram for determination of [^{14}C]ChOX species in oxidatively stressed cells is shown in Scheme 1.

2. Materials

2.1. Chemicals and reagents

All chemicals and reagents should be of the highest purity available. Unlabeled Ch, 7α-OH, 7=O, Chelex-100, desferrioxamine, 8-hydroxyquinoline, ascorbic acid, fetal bovine serum, cell growth media, and media supplements are available from Sigma Chemical Co. (St. Louis, MO). [4-^{14}C]Ch (~50 mCi/mL in toluene) is obtained from Amersham Life Sciences (Arlington Heights, IL). 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC) and 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC) are obtained from Avanti Polar Lipids (Birmingham, AL) and 5,6 > O from Steraloids (Wilton, NH). Mobile phases for TLC separations are prepared using HPLC-grade solvents. 5α-OOH and 7α-OOH, which are not commercially available, are prepared by dye-sensitized photoperoxidation of Ch in pyridine or in liposomal form [9,12]. The hydroperoxides are isolated by reverse-phase HPLC, subsequent normal-phase HPLC being used to separate 7α-OOH from any 7β-OOH [11,12]. After confirmation of identity by proton NMR [14], 5α-OOH and 7α-OOH are stored in isopropanol at −20 °C. Immediately before experiments, peroxide levels of ChOOH preparations are determined by iodometric analysis [9]. Ultrapure Millipore quality water is used for all aqueous solutions. Before use, buffer

Download English Version:

https://daneshyari.com/en/article/1211900

Download Persian Version:

https://daneshyari.com/article/1211900

Daneshyari.com