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Review

7-Hydroxycholestrol as a possible biomarker of cellular lipid peroxidation: Difference between cellular and plasma lipid peroxidation

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ABSTRACT

Polyunsaturated fatty acids and their esters are known to be susceptible to free radical-mediated oxidation, whereas cholesterol is thought to be more resistant to oxidation. In fact, it has been observed that in the case of plasma lipid peroxidation, the amount of oxidation products of polyunsaturated fatty acids such as linoleic acid was higher than that of cholesterol. In contrast, during oxidative stress-induced cellular lipid peroxidation, oxidation products of cholesterol such as 7-hydroxycholesterol (7-OHCh) were detected in greater amounts than those of linoleates such as hydroxyoctadecadienoic acid (HODE). There are several forms of oxidation products of cholesterol and linoleates *in vivo*, namely, hydroperoxides, as well as the hydroxides of both the free and ester forms of cholesterol and linoleates. To evaluate these oxidation products, a method used to determine the lipid oxidation products after reduction and saponification was developed. With this method, several forms of oxidation products of cholesterol and linoleates are measured as total 7-OHCh (t7-OHCh) and total HODE (tHODE), respectively. During free radical-mediated lipid peroxidation in plasma, the amount of tHODE was 6.3-fold higher than that of t7-OHCh. In contrast, when Jurkat cells were exposed to free radicals, the increased amount of cellular t7-OHCh was 5.7-fold higher than that of tHODE. Higher levels of t7-OHCh than those of tHODE have also been observed in selenium-deficient Jurkat cells and glutamate-treated neuronal cells. These results suggest that, in contrast to plasma oxidation, cellular cholesterol is more susceptible to oxidation than cellular linoleates. Collectively, cholesterol oxidation products at the 7-position may be a biomarker of cellular lipid peroxidation.

Contents

Abbreviations: AIPH, 2,2′-azobis[2-(2-imidazolin-2-yl)propane] dihydrochloride; Ch, cholesterol; CE-OH, cholesteryl ester hydroxide; CE-OOH, cholesteryl ester hydroperoxide; GSH, glutathione; GPx, glutathione peroxidase; HODE, hydroxyoctadecadienoic acid; HPODE, hydroperoxyoctadecadienoic acid; L, linoleates; LDL, low density lipoprotein; PBS, phosphate-buffered saline; PC-OH, phosphatidylcholine hydroxide; PC-OOH, phosphatidylcholine hydroperoxide; PH-GPx, phospholipid hydroperoxide glutathione peroxidase; PLPC, 1-palmitoyl-2-linoleoyl-sn-glycero-3-phosphatidylcholine; PUFA, polyunsaturated fatty acid; Sec, selenocysteine; TR, thioredoxin reductase; 7-KCh, 7-ketocholesterol; 7-OHCh, 7-hydroxycholesterol; 7-OOHCh, 7-hydroperoxicholesterol.

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1. Introduction

Lipid peroxidation has been the subject of extensive studies for several decades, and its mechanism, dynamics, and products are well described [1]. Lipid peroxidation produces potentially toxic compounds, but it has also been demonstrated that lipid peroxidation products may act as signaling mediators and induce an adaptive response [2,3]. It is well known that polyunsaturated fatty acids (PUFA) and their esters are vulnerable to oxidation and that their susceptibility to oxidation increases with an increase in the number of double bonds [1]. Cholesterol, another important lipid *in vivo*, is oxidized to produce versatile products termed oxysterols [4]. PUFA and cholesterol are oxidized both enzymatically and non-enzymatically; however, cholesterol, unlike PUFA, does not possess bis-allylic hydrogens and has been considered to be less susceptible to free radical-mediated oxidation than polyunsaturated lipids [5].

Lipid hydroperoxides, the primary products of lipid peroxidation, are not stable end products. Instead, they are good substrates for many enzymes such as glutathione peroxidases (GPx) and phospholipases, and they also undergo non-enzymatic secondary reactions [1,6]. It should be noted that the amount of lipid peroxidation products found in biological fluids and tissues depends on the rates of metabolism and clearance, as well as formation. Therefore, consideration of the metabolism of oxidized cholesterol and linoleates is important to the understanding of lipid peroxidation *in vivo*. Yoshida et al. have developed a method to measure the levels of lipid peroxidation *in vivo*, where total 7-hydroxycholesterol (t7-OHCh) and total hydroxyoctadecadienoic acid (tHODE) can be determined from biological samples after reduction with triphenylphosphine and saponification by potassium hydroxide [7,8]. The cholesterol oxidation products, which are oxidized at the 7-position, such as 7β-hydroxy- (7β-OHCh), 7α- and 7β-hydroperoxy-, and 7-ketocholesterol (7-KCh) are generated by non-enzymatic oxidation, whereas 7α-hydroxycholesterol (7α-OHCh) is generated by both enzymatic and non-enzymatic oxidation [4]. In this assay with reduction and saponification, oxysterols such as 7-hydroperoxycholesterol (7-OOHCh) and 7-OHCh are measured as t7-OHCh. tHODE is the sum of the following four isomers: 9- and 13-(Z,E)-HODEs; and 9- and 13-(E,E)-HODEs. Oxidation by 12/15-lipoxygenase proceeds via regio-, stereo-, and enantio-specific mechanisms to yield 13S-hydroperoxy-9Z, 11E-octadecadienoic acid (13(S)-(Z,E)-HPODE) exclusively, whereas singlet oxygen-mediated oxidation yields 9-, 10-, 12-, and 13-(Z, E)-HPODE. In contrast, oxidation of linoleates induced by free radicals yields all of the four isomers, namely, 9- and 13-(Z,E)- and 9- and 13-(E,E)-HPODEs as the primary products. HPODEs are readily reduced *in vivo* by reducing enzymes to give HODE. Therefore, 9- and 13-(E,E)-HODE are generated specifically when using free radical-mediated oxidation [9].

2. Plasma lipid peroxidation

It has been known that cholesterol is less susceptible to free radical-mediated oxidation than PUFAs such as linoleic acid. In fact, it has been reported that during low-density lipoprotein (LDL) oxidation, cholesterol is oxidized only after most PUFA esters have been oxidized [5]. It has also been reported that in the case of plasma lipid peroxidation initiated by free radical exposure, the amount of tHODE generated was 6.3-fold higher than that of t7-OHCh [10]. In a previous study, the amount of tHODE measured in free radical-exposed plasma was nearly identical to the sum of phosphatidylcholine hydroxide (PC-OH) and hydroperoxide (PC-OOH); and cholesteryl ester hydroxide (CE-OH) and hydroperoxide (CE-OOH), suggesting that the linoleates in PC and CE are the major sources of tHODE. Human plasma contains more CE than cholesterol; the ratio of CE to cholesterol is 4.1 [10]. Based on this report, the ratio of total cholesterol (tCh) to total linoleates (tL) with reduction and saponification of plasma lipids was calculated to be 4.36 (Table 1). In the plasma, when lipid peroxidation was induced by free radical exposure, the ratio of t7-OHCh to tHODE was calculated to be 0.16. Therefore, the molar relative oxidizability of plasma cholesterol to linoleates, (t7-OHCh/tHODE)/(tCh/tL), was calculated to be 0.04. These values are shown in Table 1 and Fig. 1 to compare the difference between plasma and cellular lipid peroxidation.

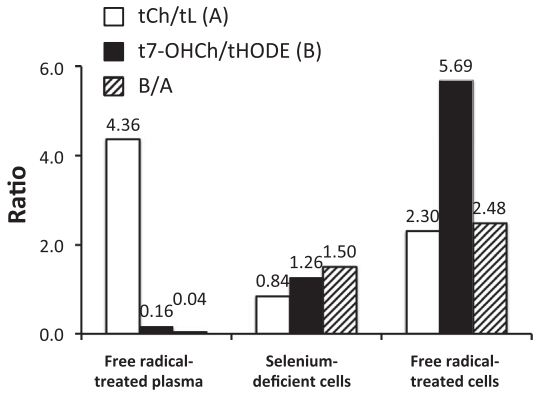

Fig. 1. The ratio of lipid peroxidation products and their substrates in plasma and cells. The ratios of total cholesterol (tCh) and total linoleates (tL) with reduction and saponification in each sample (A), t7-OHCh and tHODE obtained from each oxidized sample (B) were plotted. The molar relative oxidizability of cholesterol to linoleates obtained from (t7-OHCh/tHODE)/(tCh/tL) was also plotted (B/A).

Table 1
The amounts and the ratios of lipid peroxidation products and these substrates in plasma and cells.[a]

	Free radical-treated plasma [10]		Selenium-deficient Jurkat cells [15]	Free radical-treated Jurkat cells [15]	Glutamate-treated neuronal cells [19]	Free radical-treated erythrocytes [10]		PLPC and FC (model compounds) [10]		
	In PBS	In tBuOH/ACN				In PBS	In MeOH	In benzene	In tBuOH/ACN	In MeOH
tCh[b]	6.54	6.54	12.8	24.6	109.00	4.8	4.8	5	5	5
tL[b]	1.50	1.50	15.2	10.7	–	1.1	1.1	5	5	5
tCh/tL	4.36	4.36	0.84	2.30	–	4.4	4.4	1.0	1.0	1.0
t7-OHCh[c]	–	–	22.5	1133.00	43.00	–	–	–	–	–
tHODE[c]	–	–	17.8	199.00	0.15	–	–	–	–	–
t7-OHCh/tHODE	0.16	0.83	1.26	5.69	286.67	3.70	3.70	0.09	0.29	0.45
(t7-OHCh/tHODE)/(tCh/tL)	0.04	0.19	1.50	2.48	–	0.85	0.85	0.09	0.29	0.45

[a] Each data is referred from indicated Ref. in each column.
[b] mM in plasma, erythrocytes, and model compounds; nmol/mg protein in cells.
[c] pmol/mg protein.

3. Cellular lipid peroxidation

3.1. Selenium-deficient Jurkat cells

Selenium is an essential trace element for humans and many other species, and selenium deficiency induces pathological conditions such as cancer, coronary heart disease, and liver necrosis [11]. Selenium is an essential component of several enzymes such as glutathione peroxidase (GPx), thioredoxin reductase (TR), and selenoprotein P, which contain selenium in the form of selenocysteine (Sec) [12]. To date, 25 genes encoding Sec-containing proteins (i.e., selenoproteins) have been identified in the human genome [12]. It is well known that selenium is essential for cell culture when a serum-free medium is used [13]. Without selenium, the cells can neither proliferate nor survive. In a previous study using human T lymphoma Jurkat cells, cell death mechanisms and oxidative stress induced by selenium deficiency were investigated [14]. It was demonstrated that lipid hydroperoxides play a causative role in the oxidative damage of selenium-deficient Jurkat cells in which activities of cellular GPx, phospholipid hydroperoxide GPx (PH-GPx), and TR decrease [14]. The unexpected results demonstrated that the levels of 7-OOHCh in selenium-deficient cells increased significantly, while the levels of phospholipid hydroperoxides such as PC-OOH were below the detection limit and were lower than that of 7-OOHCh. To elucidate the effect of selenium deficiency in Jurkat cells, the lipid peroxidation products were further analyzed by t7-OHCh and tHODE methods [15]. When cells were cultured in selenium-deficient medium for 24 h, a significant increase of t7-OHCh and tHODE was observed. In the case of selenium-deficient cells, the increase in the amount of t7-OHCh (22.5 pmol/mg protein) was slightly higher than that in the amount of tHODE (17.8 pmol/mg protein). Cholesteryl ester was not detected in selenium-deficient and selenium-sufficient cells. The values from the analysis of relative susceptibility of cellular cholesterol to linoleates is as follows: tCh/tL = 0.84, t7-OHCh/tHODE = 1.26, and the molar relative oxidizability of cellular cholesterol to linoleates (t7-OHCh/tHODE)/(tCh/tL) = 1.50 (Fig. 1 and Table 1). These results suggest the preferential generation of cholesterol oxidation products in selenium-deficient Jurkat cells.

3.2. Free radical-treated Jurkat cells

To further understand the differences between cellular and plasma lipid peroxidation, Jurkat cells were treated with the water-soluble radical initiator, 2,2′-azobis[2-(2-imidazolin-2-yl)propane] dihydrochloride (AIPH), and the lipid peroxidation products were analyzed [15]. The levels of t7-OHCh and tHODE increased significantly after treatment with AIPH. The increase in the level of t7-OHCh (1130 pmol/mg protein) was 5.7-fold greater than that of tHODE (199 pmol/mg protein). The molar relative susceptibility of cellular cholesterol to linoleates is as follows: tCh/tL = 2.30, t7-OHCh/tHODE = 5.69, (t7-OHCh/tHODE)/(tCh/tL) = 2.48 (Fig. 1 and Table 1). These results imply that cholesterol is more susceptible than linoleates to free radical-induced peroxidation in cultured cells. In the case of erythrocytes treated with the radical initiator AIPH, a similar preferential generation of cholesterol oxidation products has been reported [10]. The values of the erythrocytes are shown in Table 1.

3.3. Glutamate-treated neuronal cells

Glutamate, the predominant excitatory neurotransmitter in the mammalian brain, is a major contributor to neuronal cell death under pathological conditions such as epilepsy, ischemic insults, and traumatic brain damage [16]. Exposure of cells to glutamate causes inhibition of cystine transport into the cells, which results in an inability to maintain intracellular glutathione (GSH) levels [17]. The low levels of intracellular GSH lead to a reduced ability to protect cells against oxidative stress and ultimately induces cell death. The generation of excess free radicals might be responsible, at least in part, for cell toxicity because cell death can be prevented by the administration of various free radical-scavenging antioxidants, including vitamin E [18,19]. Lipid oxidation products in the glutamate-treated neuronal cells were also analyzed using the t7-OHCh and tHODE methods [19]. In the glutamate-treated neuronal cells, a significant increase in oxidation products of cholesterol was observed, and the amount of t7-OHCh in glutamate-treated cells increased to 43 pmol/mg protein. However, a significant generation of oxidation products of linoleates was not observed. The values of glutamate-treated neuronal cells are shown in Table 1.

4. Difference between cellular and plasma lipid peroxidation

These lines of evidence suggest that cellular cholesterol is more susceptible to oxidation than plasma cholesterol. To understand these observations chemically, the relative susceptibility of cholesterol and linoleates to free radical-medicated oxidation in several organic solution was investigated [10]. Using 1-palmitoyl-2-linoleoyl-sn-glycero-3-phosphatidylcholine (PLPC) and free cholesterol as model compounds, it was shown that the chemical oxidizability of cholesterol and linoleates is highly dependent on the kind of solvent [10]. The oxidation of 5 mM PLPC and 5 mM cholesterol by a radical initiator in a benzene solution resulted in the preferential generation of tHODE, whereas choletserol was not effectively oxidized in benzene (Table 1). In contrast, oxidation of PLPC and cholesterol in acetonitrile/*tert*-butyl alcohol (4/1 by volume) or methanol resulted in an increase in both tHODE and t7-OHCh. The ratios of t7-OHCh to tHODE in each solution were as follows: benzene, 0.09; acetonitrile/*tert*-butyl alcohol, 0.29; and methanol, 0.45 (Table 1) [10].

In the case of free radical-medicated oxidation of total lipids extracted from plasma in the acetonitrile/*tert*-butyl alcohol, both t7-OHCh and tHODE increased in accordance with the oxidation of model compounds in acetonitrile/*tert*-butyl alcohol (Table 1). The oxidation of plasma lipids in phosphate-buffered saline (PBS) resulted in a marked increase in tHODE levels, but t7-OHCh levels were not increased. Collectively, these results suggest that plasma lipid peroxidation in physiological conditions such as PBS preferentially generate tHODE, whereas the oxidation of lipids extracted from plasma in an organic solvent could generate the oxidation products of cholesterol and linoleates. Therefore, we propose that the structure of lipoproteins in the physiological conditions might increase the preferential oxidation of linoleates over cholesterol [10].

In the case of cellular lipid peroxidation, oxidation of total lipids extracted from erythrocytes in methanol resulted in a marked increase in t7-OHCh in accordance with the results of oxidation of erythrocytes in PBS. At present, the molecular mechanisms that explain the difference between cellular and plasma lipid peroxidation remain to be elucidated. However, these observations imply that the lipid peroxidation *in vivo* may be, at least in part, influenced by the milieu where the substrates exist, because the oxidizability of cholesterol and linoleates is highly dependent on the solvent.

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