Adaptive response induced by lipid peroxidation products in cell cultures

Zhi-Hua Chen\textsuperscript{a,*}, Yasukazu Yoshida\textsuperscript{a}, Yoshiro Saito\textsuperscript{a}, Noriko Noguchi\textsuperscript{b,c}, Etsuo Niki\textsuperscript{a}

\textsuperscript{a} Human Stress Signal Research Center (HSSRC), National Institute of Advanced Industrial Science and Technology (AIST), 1-8-31, Midorigaoka, Ikeda, Osaka 563-8577, Japan
\textsuperscript{b} Research Center for Advanced Science and Technology, University of Tokyo, 4-6-1, Komaba, Tokyo 153-8904, Japan
\textsuperscript{c} Science and Engineering Research Institute, Doshisha University, 1-3 Miyakotani, Tatara, Kyotanabe, Kyoto 610-0394, Japan

Received 20 October 2005; revised 28 November 2005; accepted 16 December 2005

Available online 22 December 2005

Edited by Vladimir Skulachev

Abstract The adaptive response induced by the lipid peroxidation products, such as phosphatidylcholine hydroperoxide, lysophosphatidylcholine (LysoPC), 15-deoxy-\(\Delta^{12,14}\)-prostaglandin J\(_2\), 4-hydroxynonenal (4-HNE), hydroxyoctadecadienoic acid, 7-hydroxycholesterol, and cholesterol 5\(\beta\),6\(\beta\)-epoxide, was investigated in this study. Although these products have been implicated in oxidative stress-related diseases, pretreatment with such compounds at sublethal concentrations significantly protected PC12 cells against subsequent oxidative stress induced by 6-hydroxydopamine. Moreover, 4-HNE and LysoPC also exhibited adaptive protection in human arterial endothelial cells. These findings suggest a general hormetic effect of such compounds in cell cultures and may lead to a reappraisal of the eventual role of reactive oxygen species and lipid peroxidation in organisms.

\(\copyright\) 2005 Federation of European Biochemical Societies. Published by Elsevier B.V. All rights reserved.

Keywords: Lipid peroxidation products; Adaptive response; Oxidative stress; Cytotoxicity

1. Introduction

The generation of reactive oxygen species (ROS) and subsequent oxidative modification of biomolecules, such as lipids, proteins, and nucleic acids, are inevitable in aerobic organisms. An excessive amount of ROS, referred to as oxidative stress, has been implicated in a variety of pathological events such as atherosclerosis, ischemia-reperfusion injury, cardiovascular diseases, and neurodegenerative diseases [1]. Above all, lipid peroxidation has received much attention and has been accepted to cause disturbance of fine structure and functional loss of biological membranes and to produce some toxic products.

Phosphatidylcholine hydroperoxide (PCOOH) is the primary product of lipid peroxidation, which undergoes non-enzymatic reactions, leading to the formation of 4-hydroxynonenal (4-HNE) and malondialdehyde, secondary products of lipid peroxidation [2,3]. Alternatively, the lipid hydroperoxides, especially linoleic acid hydroperoxide, can also be oxidized enzymatically by some peroxidases and reductases into hydroxyoctadecadienoic acids (HODEs) [4,5]. Lysophosphatidylcholine (lysoPC) is generated from phosphatidylcholine during oxidative modification of low density lipoprotein (LDL) [6,7]. Prostaglandins of the J series are cyclopentenones synthesized from arachidonic acid via enzymatic conversion by cyclooxygenase and prostaglandin D\(_2\) synthase, followed by the non-enzymatic dehydration from prostaglandin D\(_2\) to the J series, including 15-deoxy-\(\Delta^{12,14}\)-prostaglandin J\(_3\) (15d-PGJ\(_3\)) [8]. Oxysterols are defined as oxygenated derivatives of cholesterol that may be formed directly by autoxidation or by the action of a specific mono-oxygenase [9].

It is widely accepted that all these lipid peroxidation products mentioned above could induce oxidative stress and be involved in the pathogenesis of a number of degenerative diseases [10–13], or have been considered as biomarkers of oxidative stress [14]. However, recent studies have revealed that low levels of ROS and certain lipid oxidation products may play essential roles in the cell signal transduction [15,16] and can induce adaptive response [17–23]. We were therefore interested in studying whether these physiologically existed lipid peroxidation products could also induce adaptive response at low concentrations. We have recently found that stimulation with sublethal concentrations of H\(_2\)O\(_2\) or 4-HNE can induce adaptive response and protect neural PC12 cells against the subsequent oxidative stress [19,24], whereas little is known about the possible adaptive effect induced by others of these lipid peroxidation products. Therefore, in the present study, in order to explore the generality of this phenomenon, we attempted to examine the possible adaptive response to these lipid peroxidation products in neuronal PC12 cells. We found that stimulation of PC12 cells with these compounds at sublethal concentrations induced adaptive response and protected cells against the subsequent oxidative stress induced by 6-hydroxydopamine (6-OHDA), which has been demonstrated to induce oxidative damage in neural system and implicated in neurodegenerative disorders [25]. The possible adaptive response induced by 4-HNE and lysophosphatidylcholine (LysoPC) in human arterial endothelial cells (HAECs) has also been investigated.

\(\beta\)-EPOX-Ch, cholesterol 5\(\beta\),6\(\beta\)-epoxide; 15d-PGJ\(_2\), 15-deoxy-\(\Delta^{12,14}\)-prostaglandin J\(_2\); 4-HNE, 4-hydroxynonenal; HODE, hydroxyoctadecadienoic acid; LysoPC, lysophosphatidylcholine; 7-OHCh, 7-hydroxycholesterol; 6-OHDA, 6-hydroxydopamine; PCOOH, phosphatidylcholine hydroperoxide; ROS, reactive oxygen species

*Corresponding author. Fax: +81 72 751 9964.
E-mail address: zhihua-chen@aist.go.jp (Z.-H. Chen).

Abbreviations: \(\beta\)-EPOX-Ch, cholesterol 5\(\beta\),6\(\beta\)-epoxide; 15d-PGJ\(_2\), 15-deoxy-\(\Delta^{12,14}\)-prostaglandin J\(_2\); 4-HNE, 4-hydroxynonenal; HODE, hydroxyoctadecadienoic acid; LysoPC, lysophosphatidylcholine; 7-OHCh, 7-hydroxycholesterol; 6-OHDA, 6-hydroxydopamine; PCOOH, phosphatidylcholine hydroperoxide; ROS, reactive oxygen species.
2. Materials and methods

2.1. Materials
LysoPC was purchased from Wako, Osaka, Japan. 4-HNE and 15d-PGJ$_2$ were from Calbiochem Co., CA, USA. Authentic 7α- and 7β-hydroxysterol (OHCh) were obtained from Steraloids Inc., Newport, RI, USA. 9-EE-, 9-EZ-, 13-EE-HODE were products of Cayman Chemical Company, MI, USA. PCOOH was a kind gift from Dr. Takahashi in Hokkaido Pharmaceutical University. 6-OHDA and cholesterol 5β,6β-epoxide (β-EPOX-Ch) were obtained from Sigma Chemical Company. 3(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) was from Djinodo Kumamoto, Japan. 2′,7′-Dichlorofluoroxcin diacetate (DCFH-DA) was purchased from Molecular Probes (Eugene, OR, USA). All other chemicals were of the highest quality commercially available.

2.2. Cell culture and determination of cell viability
PC12 cells were routinely cultured in DMEM/F-12 medium with l-glutamine, sodium bicarbonate and pyridoxine hydrochloride, supplemented with 10% fetal bovine serum and 5% horse serum. HAECs were cultured in EBM-2 medium containing 2% (v/v) fetal bovine serum, 10 ng/mL human epidermal growth factor, 5 ng/mL fibroblast growth factor, 10 µg/mL heparin, 1 µg/mL hydrocortisone, 0.5 µg/mL gentamycin and 50 µg/mL amphotericin B according to the manufacturer’s instruction. Both cell cultures were maintained at 37 °C in a humidified atmosphere of 5% CO$_2$.

For determination of the cell viability, the conventional MTT reduction assay was used. The treated cells were incubated with 0.5 mg/ml MTT at 37 °C for 1 h. Isopropyl alcohol containing 0.04 N HCl was added to the culture medium (3:2 by volume) and mixed with a pipette until the formazan was completely dissolved. The optical density of formazan was measured at 570 nm using a Multiskan Ascent plate reader (Thermo Labsystems, Helsinki, Finland).

For the adaptive response study, cells were pretreated with sublethal concentrations of oxidative stimuli for 24 h. The culture medium was then removed and fresh medium containing different concentrations of second stressors was added into the cell cultures. The cells were then incubated for additional 24 h and followed with the MTT assay to check the cell viability.

2.3. Determination of intracellular ROS
Intracellular ROS were detected using DCFH-DA, as described previously [19]. Following treatment and DCFH-DA staining, cells were excited with a 488 nm argon ion laser in a Cytomics FC500 Flow Cytometry system and DCF emission was recorded at 525 nm. Data were collected from at least 10000 events.

2.4. Statistics
All data of at least three independent experiments are expressed as means ± S.D. and analyzed by the Student’s t test. Values of $P < 0.05$ were considered to be statistically significant.

3. Results

3.1. Cytotoxicity induced by various lipid peroxidation products in PC12 cells
In order to explore the possible adaptive response by these lipid peroxidation products, the cytotoxicity induced by such compounds was first investigated in PC12 cells. As shown in Fig. 1, all listed compounds induced significant cell death at high concentrations, whereas the three isomers of HODE at concentrations till 50 µM did not exhibit any cytotoxicity (data not shown).

![Fig. 1. Cytotoxicity induced by lipid peroxidation products in PC12 cells. Cells were treated with each compound at various concentrations for 24 h, and the cell viability was determined by MTT assay as described in Section 2.](image)

![Fig. 2. Adaptive response induced by various kinds of lipid peroxidation products in PC12 cells. (A) Adaptive cytoprotection induced by primary lipid peroxidation products, LysoPC and PCOOH at sublethal concentrations. (B) Adaptive cytoprotection induced by the further degenerated lipid peroxidation products, HODE and 15d-PGJ$_2$ at sublethal concentrations. (C) Adaptive protection induced by oxysterols, 7α-OHCh, 7β-OHCh and β-EPOX-Ch at sublethal concentrations. PC12 cells were pretreated with or without these lipid peroxidation products for 24 h, and followed by the treatment with various concentrations of 6-OHDA for an additional 24 h. *Significantly different from the corresponding value without pretreatment ($P < 0.05$).](image)
3.2. Adaptive cytoprotection induced by the lipid peroxidation products in PC12 cells

We then studied the possible adaptive response induced by these lipid peroxidation products. Based on the above findings, the corresponding sublethal concentrations at which the lipid peroxidation product did not induce any cytotoxicity were then selected for studying the possible adaptive response induced by this compound. The pretreatment of PC12 cells with sublethal concentrations of 15d-PGJ$_2$ or 7α-OHCh significantly protected cells against the cytotoxicity induced by themsevles (data not shown), suggesting an adaptive response was induced by these oxidative stimuli. Furthermore, pretreatment of PC12 cells with the primary lipid peroxidation products, LysoPC (125 μM) or PCOOH (10 μM) (Fig. 2A), or further degenerated lipid peroxidation products, HODE (50 μM) or 15d-PGJ2 (7.5 μM) (Fig. 2B), or oxysterols, 7-hydroxycholesterol (7-OHCh, 20 μM) or β-EPOX-Ch (15 μM) (Fig. 2C), for 24 h significantly protected cells against the cytotoxicity induced by 6-OHDA.

3.3. Intracellular ROS accumulation induced by 6-OHDA was effectively attenuated by the lipid peroxidation products at their adaptive concentrations

The levels of ROS accumulation reflect the intracellular redox status. We then investigated the possible effect of these lipid peroxidation products on the basal level and the 6-OHDA-induced accumulation of ROS. Pretreatment of these lipid peroxidation products at sublethal concentrations did not cause any appreciable variation on the basal level of ROS (data not shown), however, the pretreatment effectively attenuated the ROS accumulation induced by 6-OHDA (Fig. 3). In the unpretreated cells, incubation of 50 μM 6-OHDA for 6 h significantly induced the ROS accumulation till approximately 2.5-fold, which was effectively attenuated, even completely abolished, by the pretreatment of sublethal lipid peroxidation products, suggesting the cellular antioxidant capacity was enhanced by the pretreatment of these lipid peroxidation products.

3.4. Adaptive response induced by 4-HNE and LysoPC in HAECs

In order to study whether these lipid peroxidation products could also induce adaptive effect in endothelial cells, we then investigated the possible adaptive response in HAECs. As shown in Fig. 4, both 4-HNE and LysoPC at concentrations higher than 5 μM induced significant cell death (Fig. 4A), and pretreatment with either of these compounds at 5 μM for 24 h significantly protected HAECs against the cytotoxicity induced by 7β-OHCh (Fig. 4B), which has been demonstrated to be involved in coronary artery disease [13].

4. Discussion

It has been widely accepted that low levels of H$_2$O$_2$ and related ROS play essential roles in the cell signal transduction [16] and can induce adaptive response [17–22]. Such roles of some other physiological oxidative stimuli or oxidation products in modulating and participating in signal transduction have received increased attention. For instance, 4-HNE, one of the major end products of lipid peroxidation and previously known as an oxidative agent, now has been recognized as a signaling molecule at low concentrations and considered to bridge the fields of oxidative stress and redox signaling [24,26,27]. Likewise, cyclopentenone prostaglandins, which have shown the proapoptotic effect in a variety of cell types, now have been reported to exert protective effect at low concentrations via different mechanisms [28–30]. In the present study, we clearly demonstrated that other lipid peroxidation products, such as LysoPC, PCOOH, 7-OHCh, β-EPOX-Ch and HODE, could also induce adaptive response at sublethal concentrations, protecting PC12 cells against the subsequent effects.

---

**Fig. 3.** Pretreatment of the sublethal concentration of lipid peroxidation products effectively attenuated the 6-OHDA-induced ROS accumulation in PC12 cells. Cells were pretreated with or without the lipid peroxidation products for 24 h, and followed by the incubation of 50 μM 6-OHDA for an additional 6 h. Cells were then stained with 5 μM DCFH-DA for 30 min and harvested for the ROS analysis, as described in Section 2. *Significantly different from the value of control (P < 0.05). #Significantly different from the corresponding value without pretreatment (P < 0.05).
The detailed molecular mechanisms for such an adaptive response might be specific to a given cell type as well as to a given stimulus. For example, it has been reported that H₂O₂-induced adaptive response was mediated through either increased catalase and glutathione peroxidase (GPx) activities in PC12 cells [19], or enhanced catalase, GPx, and CuZnSOD activities in the adrenal cortex cells [20], or elevated GSH contents in myocardial cells [21], or upregulated thioredoxin-1 in human umbilical vein endothelial cells [22]. We have recently reported that 4-HNE-induced adaptive response in PC12 cells was critically mediated through induction of thioredoxin reductase-1 (TR-1) via transcriptional activation of Nrf2 [24]. Interestingly, other lipid peroxidation products, such as LysoPC, 7-OHCh, PCOOH and HODE, did not induce any considerable TR-1 expression at the adaptive concentrations (data not shown). Furthermore, we have recently found that both 7-OHCh and 15d-PGJ₂ induced significant elevation of the cellular GSH in PC12 cells and this increase might be responsible for the adaptive cytoprotection, whereas LysoPC, PCOOH, β-EPOX-Ch and HODE did not exert any effect on the GSH level (Chen et al., unpublished data). All these findings suggest, in addition to TR1 and GSH, other cellular antioxidative events may also be involved in the adaptive response, although the eventual role of which proteins or enzymes in the adaptive response needs to be further investigated.

This extensively-existed adaptive response by a variety of oxidative stimuli in various cell types may lead us to rethink the role of ROS and lipid peroxidation products in cells. Such a hormetic effect may also require a reappraisal of the functions of ROS and subsequent lipid peroxidation in organisms. Based on the present study and other previous reports, it would be quite reasonable to assume that so-called oxidative stress at the controlled or mild levels may act as a good stress, “eustress”. It would be also rational to assume that, at initial time of oxidative stress, accumulation of lipid peroxidation products serves as a feedback which stimulates the defense network and transmits a SOS signal, thereby triggering the early-response enzymes or proteins to cope with the forthcoming oxidative disaster.

In conclusion, the present study clearly shows that various lipid peroxidation products at sublethal concentrations induce adaptive response and enhance cell tolerance. Cells treated with these compounds exhibit higher resistance to oxidative stress, although the detailed underlying molecular mechanisms need to be further examined. These results represent an initial effort to demonstrate the possible adaptive effect of the lipid peroxidation products at sublethal concentrations, which may lead to a reappraisal of the eventual role of ROS and lipid peroxidation in organisms.

References


