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Lipid Hydroperoxides (LOOHs)

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Abbreviations

CAT, catalase
GPx, glutathione peroxidase
GSH, glutathione
GSSG, glutathione disulfide
LOOH, lipid hydroperoxide
ROS, reactive oxygen species
SOD, superoxide dismutases

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Abstract

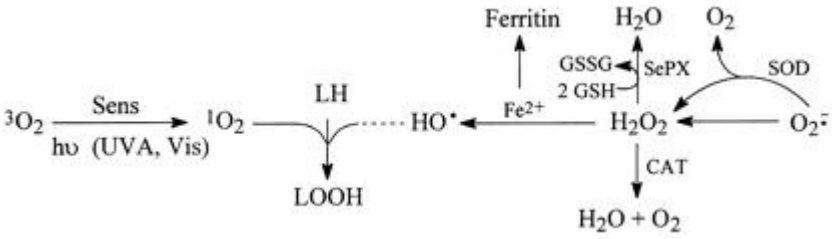
Reactive Oxygen Species (ROS) are produced during the normal cellular metabolism of oxygen. Therefore, ROS are formed constantly in our bodies and cause damage. Lipid peroxidation is the most obvious oxidative damage in cell membranes and other lipid-containing structures. Lipid peroxidation can be triggered by two types of species. One is free radical species, such as oxyl radicals and hydroxyl radicals. The other one is non-radical species, such as singlet oxygen, ozone and peroxynitrite. Lipid hydroperoxides (LOOHs) are derived from unsaturated phospholipids, glycolipids, and cholesterol. LOOHs through one-electron reduction can produce lipid alkoxyl radicals. And the alkoxyl radicals can also be the initiators of lipid peroxidation. LOOHs also cause oxidative stress to cells, which may cause cellular responses, such as evoke antioxidant enzymes activities or apoptosis.

Introduction

Tissue increased oxidative destruction and degeneration cancan result in ROS are constantly formed in the human body and removed by antioxidant defenses. For example, a large number of phagocytes become activated in a localized area, they can produce tissue damage. Cells are equipped with a variety of primary and secondary defenses against lipid peroxidation. Therefore, organisms use enzymatic means to inhibit lipid peroxidation. Such as, superoxide dismutases, catalase (CAT), and glutathione peroxidase (GPx) as protection against generation of ROS. [2]

LOOH formation and turnover

Lipid-containing structures in cell membranes are a major target of oxidant attack. For example, atherogenesis, UV-induced carcinogenesis, and ischemia-reperfusion injury are all related to lipid peroxidation. If excess ROS are not eliminated by antioxidants, they will cause oxidative damage. ROS such as $^1O_2$ or HO give rise to primary stage LOOHs. [2]



$L\text{-}H + X^{\bullet} \xrightarrow{Ri} L^{\bullet} + XH$ **Initiation**

$L^{\bullet} + O_2 \xrightarrow{ko} LOO^{\bullet}$

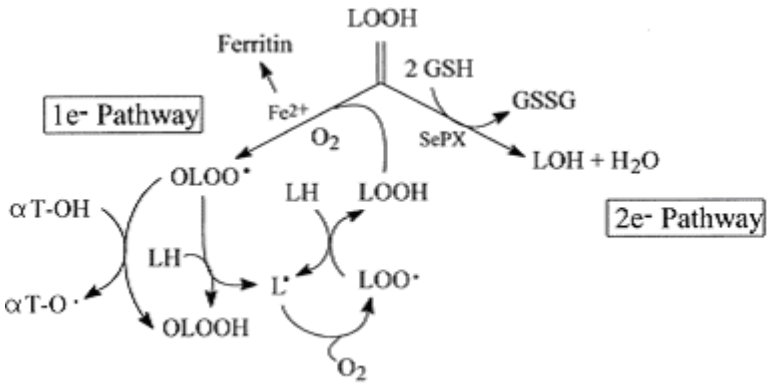
$LOO^{\bullet} + L\text{-}H \xrightarrow{kp} LOOH + L^{\bullet}$ **Propagation**

$LOO^{\bullet} + LOO^{\bullet} \xrightarrow{kt}$ NPR (non-radical product)

$LOO^{\bullet} + L^{\bullet} \longrightarrow$ NPR

$L^{\bullet} + L^{\bullet} \longrightarrow$ NPR **Termination**

These, LOOHs may also undergo two-electron reduction to redox-inert alcohols (LOHs); these reactions are typically catalyzed by GSH-dependent GPx. The LOOHs may also undergo iron-mediated (metals) reaction to give epoxyallylic peroxyl radicals ($OLOO^{\bullet}$).



If metals are present, LOOHs have chain-branching reactions. [5]

$LOOH + M^{n} \longrightarrow LO^{\bullet} + OH^{-} + M^{n+1}$ and

$LO^{\bullet} + LOOH \longrightarrow LOH + LOO^{\bullet}$

$LO^{\bullet} + LH \longrightarrow LOH + L^{\bullet}$ or

$LO^{\bullet} \longrightarrow OL^{\bullet}$ or

$OL^{\bullet} + O_2 \longrightarrow OLOO^{\bullet}$

Detection of lipid radicals

The most applied method is electron-spin-resonance (ESR). Principle idea of ESR is a spectroscopic technique to detect species that have unpaired electrons. The phosophonic acid compound, 5-(diethoxyphosphoryl)-5-methyl-1-pyrroline (DEPMPO), is one of the most suitable spin traps to detect superoxide radicals has recently been described by Frejavile *et al.* The half-life of DEPMPO/ $OOH^{\bullet}$ is about 10 times higher than 5,5-Dimethyl-1 –pyrroline *N*-oxide (DMPO) adduct. There are also several kinds of short half-life of alkoxyl radicals, such as DMPO, DEPMPO, and EMPO, spin traps are usually used. [3]

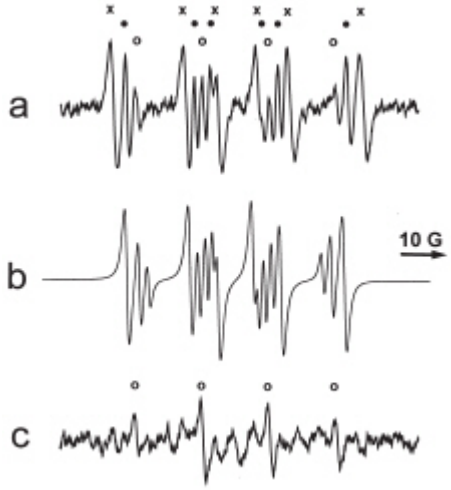


Figure 1. Lipid-derived free radical adducts and Fe^{2+} in the presence of the spin trap DMPO. a) Experimental spectrum in anaerobic system.”x” carbon-centered radicals. “•” acyl radicals. “。” hydroxyl radicals. b) Computer simulation of an aerobic system c) Aerobic system, showing only traces of DMPO/$^{\bullet}$OH

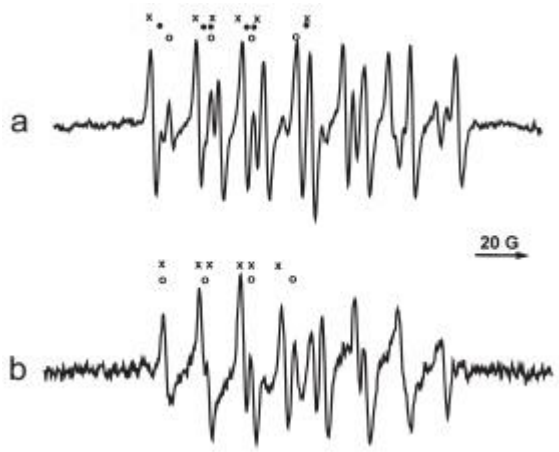


Figure 2. Lipid-derived free radical adducts and Fe^{2+} in the presence of the spin trap DPPMPO. a) anaerobic system (N_2). b) aerobic system (O_2).”x” carbon-centered radicals. “•” acyl radicals. “。” hydroxyl radicals.

To compare figure (1a) DMPO system and figure (2b) DPPMPO system, it is clearly show a second species in addition to the DPPMPO/$^{\bullet}$OH adduct. Therefore, combination of experiments using different spin traps will give a clearer picture than only use one spin trap.

The biological effects of LOOHs

Apoptosis plays a critical role in maintaining healthy tissue. To detect lipid hydroperoxides (LOOHs) -induced redox shifts in apoptosis using the human colonic CaCo-2 cell. Data shows subtoxic concentrations of LOOH increased CaCo-2 cell apoptosis. This LOOH-induced apoptosis was associated with a significant decrease in the ratio of reduced glutathione-to-oxidized glutathione (GSH/GSSG). Control cells (*A*) and cells treated for 24 h

with 10 μM LOOH (*B*) were stained by TUNEL and analyzed by flow cytometry. The results show that after exposure of cells to 10 μM LOOH the percent of TUNEL-positive cells increased to 24.6 ± 1.7%. In agarose gel electrophoresis (C) of total cell DNA shows that LOOH caused a dose-dependent increase in DNA fragmentation with significant increases at 10 μM and 20 μM LOOH. In (C), Lanes 1, 2, 3, and 4 were DNA from cells treated with LOOH concentrations at 0, 2, 10, and 20 μM, respectively. Lanes 5 and 6 were DNA markers. [5]

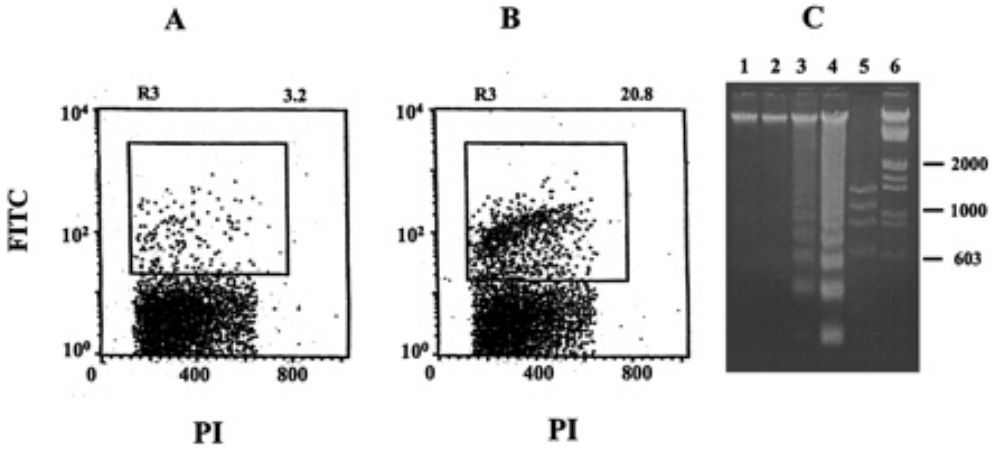


Figure 3. Analysis of apoptosis of CaCo-2 cells by flow cytometry and agarose gel electrophoresis.

The release of cytosolic LDH can be an index of cell membrane damage. After exposure of CaCo-2 cells to LOOH concentrations between 1 μM and 25 μM for 24 h, the ratio of apoptosis will increase10%–30%. LDH release was slowly between 0–10 μM LOOH. At 25 μM LOOH , LDH was significantly detected.

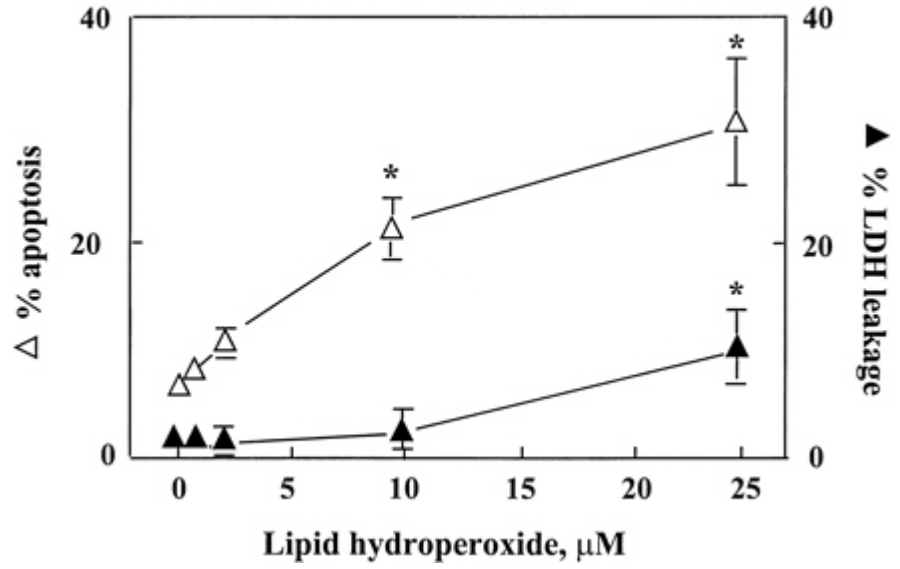


Figure 4. Effect of LOOH concentrations on CaCo-2 cell cytotoxicity and apoptosis. Cytotoxicity was determined by cytosolic release of LDH. Quantification of apoptosis was determined by TUNEL staining and flow cytometry.

Exposure of cells to different LOOH concentrations (0–25 μM) for 24 h, cell GSH concentration decreased significantly. LOOH at 0.4 μM caused a small increase in cellular GSH. That may imply mild oxidant stress promotes a compensatory increase in GSH. Another important marker is the GSH/GSSG ratio. The GSH/GSSG ratio fell at all LOOH concentrations. Maximal GSH loss and decreased GSH/GSSG ratio were achieved at 10 μM and 25 μM LOOH and was correlated with maximal cell apoptosis. The results show that treatment of cells with LOOH caused a rapid oxidation of GSH and GSH/GSSG ratio. [4]

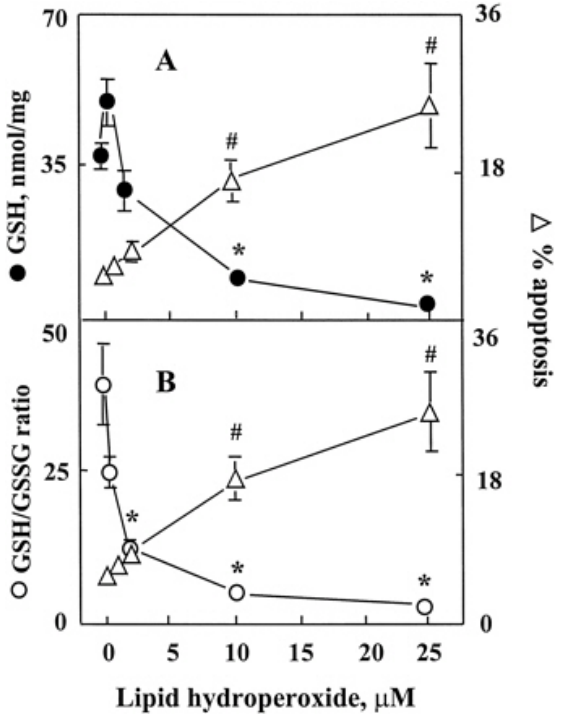


Figure 5. Relationship between LOOH-induced GSH/GSSG imbalance and apoptosis of CaCo-2 cells.

Summary

LOOHs are known agents of cytotoxicity. Cell cycle checkpoints have shown that regulate cells into death or proliferation. However, oxidants in subtoxicity contentration can play an important role in mediating specific cell responses and expression of genes. Recent studies have demonstrated that at low concentrations of LOOH (1 μM and 5 μM) promotes CaCo-2 cell proliferation and cell cycle progression. These data provide evidence to support the hypothesis that LOOH-induced oxidative stress can cause apoptosis in CaCo-2 cells and also closely linked to LOOH-induced disruption of normal cellular thiol/disulfide status. One

important observation in the data is that apoptosis in CaCo-2 cells was mediated predominantly by decreasing in the GSH/GSSG ratio rather than by changes in levels of GSH *per se*. Cell apoptosis was associated with increased GSSG relative to GSH. Another important observation is that oxidative DNA damage did not contribute to the apoptotic process. In another word, DNA oxidation occurs as a result of cells undergoing apoptosis. [3] [4]

Reference

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