review

Lipid hydroperoxide generation, turnover, and effector action in biological systems

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Abstract **Lipid peroxidation is a well known example of oxidative damage in cell membranes, lipoproteins, and other lipid-containing structures. Peroxidative modification of unsaturated phospholipids, glycolipids, and cholesterol can occur in reactions triggered by *i*) free radical species such as oxyl radicals, peroxyl radicals, and hydroxyl radicals derived from iron-mediated reduction of hydrogen peroxide or *ii*) non-radical species such as singlet oxygen, ozone, and peroxynitrite generated by the reaction of superoxide with nitric oxide. Lipid hydroperoxides (LOOHs) are prominent non-radical intermediates of lipid peroxidation whose identification can often provide valuable mechanistic information, e.g., whether a primary reaction is mediated by singlet oxygen or oxyradicals. Certain cholesterol-derived hydroperoxides (ChOOHs) have been used very effectively in this regard, both in model systems and cells. Being more polar than parent lipids, LOOHs perturb membrane structure/function and can be deleterious to cells on this basis alone. However, LOOHs can also participate in redox reactions, the nature and magnitude of which often determines whether peroxidative injury is exacerbated or prevented. Exacerbation may reflect iron-catalyzed one-electron reduction of LOOHs, resulting in free radical-mediated chain peroxidation, whereas prevention may reflect selenoperoxidase-catalyzed two-electron reduction of LOOHs to relatively non-toxic alcohols. LOOH partitioning between these two pathways in an oxidatively stressed cell is still poorly understood, but recent cell studies involving various ChOOHs have begun to shed light on this important question. An aspect of related interest that is under intensive investigation is lipid peroxidation/LOOH-mediated stress signaling, which may evoke a variety of cellular responses, ranging from induction of antioxidant enzymes to apoptotic death. Ongoing exploration of these processes will have important bearing on our understanding of disease states associated with peroxidative stress.—** Girotti, A. W. **Lipid hydroperoxide generation, turnover, and effector action in biological systems. *J. Lipid Res.* 1998.** 39: **1529–1542.**

Supplementary key words oxidative stress • lipid peroxidation • cholesterol • hydroperoxides • membranes • lipoproteins • iron • selenoperoxidases • signal transduction • apoptosis

Propagative lipid peroxidation is a degenerative process that affects cell membranes and other lipid-containing structures under conditions of oxidative stress (1–3). Our understanding of how this complex process occurs and how cells respond to it, e.g. by enhancing cytoprotective activity, is improving steadily, but much remains to be learned. The intent of this review is to address some of these important issues. Lipid hydroperoxides (LOOHs) derived from unsaturated phospholipids, glycolipids, and cholesterol are prominent intermediates of peroxidative reactions induced by activated species such as hydroxyl radical, lipid oxyl or peroxyl radicals, singlet oxygen, and peroxynitrite (2, 3). Once formed, LOOHs may undergo reductive degradation which either diminishes or enhances cytotoxic potential, depending on a variety of circumstances (3–5). In addition, LOOHs or related peroxidation intermediates/products may trigger signal transduction pathways calling for either greater cytoprotection (exemplified by up-regulation of detoxifying enzymes) or deliberate termination (apoptotic death) (6). Although much has been written about lipid peroxidation and its possible cytopathological consequences (1–3), less attention has been directed to LOOHs per se, e.g., mechanisms

Abbreviations: ROS, reactive oxygen species; PUFA, polyunsaturated fatty acid; LOOH, lipid hydroperoxide; LO•, lipid oxyl radical; LOO•, lipid peroxyl radical; OLOO•, epoxyallylic peroxyl radical; PLOOH, phospholipid hydroperoxide; ChOOH, cholesterol hydroperoxide; 5α-OOH: 3β-hydroxy-5α-cholest-6-ene-5-hydroperoxide; 6α-OOH: 3-hydroxycholest-4-ene-6α-hydroperoxide; 6β-OOH: 3β-hydroxycholest-4-ene-6β-hydroperoxide; 7α,7β-OOH: mixture of 3β-hydroxycholest-5-ene-7α-hydroperoxide and 3β-hydroxycholest-5-ene-7β-hydroperoxide; 5,6-epoxide: unspecified mixture of 5,6α-epoxy-5α- and 5,6β-epoxy-5β-cholestan-3β-ol; $Fe(HQ)_2$, ferric-8-hydroxyquinoline; DFO, desferrioxamine; TBARS, thiobarbituric acid reactive substances; SePX, selenoperoxidase; GPX, glutathione peroxidase; PHGPX, phospholipid hydroperoxide glutathione peroxidase; SOD, superoxide dismutase; CAT, catalase; GSH/GSSG, reduced glutathione/glutathione disulfide; PLA_2, phospholipase-A_2; Se(+), selenium-replete cells; Se(−), selenium-deficient cells; HPLC-EC(Hg), high performance liquid chromatography with mercury cathode electrochemical detection; TLC, thin-layer chromatography.

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of formation under various prooxidant conditions, and comparative susceptibilities to one-electron or two-electron reduction in cells. This article deals with these aspects for LOOHs in general, but with some emphasis on cholesterol-derived hydroperoxides (ChOOHs), which arise most prominently in plasma membranes and lipoproteins, and have been studied extensively in this laboratory (4). Recent advances in this area have been made possible largely through the development of highly sensitive and specific methods for measuring LOOHs, some of which will be mentioned along the way.

OXYGEN ACTIVATION, LIPID PEROXIDATION, AND LIPID HYDROPEROXIDES

Reactive oxygen species

Small amounts of potentially toxic reactive oxygen species (ROS) can be generated in eukaryotic cells by normal oxidase action and during the course of electron transport in mitochondria or endoplasmic reticulum (3, 7). Electron "leaks" in the transport pathways can give rise to superoxide radical (O_2^-), which may either disproportionate or be reduced, giving rise to hydrogen peroxide (H_2O_2). The generation rate of these ROS can increase dramatically when cells are confronted with redox cycling compounds such as bipyridyls or anthracyclines, which intercept electrons at the level of flavins or quinone carriers and then autoxidize, resulting in large increases in O_2^- and H_2O_2 yield (8–10). In the special case of phagocytic cells (macrophages, neutrophils), O_2^- is deliberately overproduced during an "oxidative burst" associated with NADPH oxidase activation, and H_2O_2 is correspondingly generated (11). While O_2^- and H_2O_2 may be individually damaging under some circumstances (12, 13), the effects are usually more dramatic in combination with one another or with other reactive species. Perhaps the best known example is iron-mediated reduction of H_2O_2 by O_2^- (reduction of Fe^{3+} by O_2^- coupled to Fenton-type reduction of H_2O_2 by Fe^{2+}), which gives rise to hydroxyl radical ($HO^\bullet$), an exceedingly strong and indiscriminate oxidant that can abstract allylic hydrogens, add (hydroxylate), or accept electrons, depending on the target molecule (14). Most $HO^\bullet$ reactions are extremely rapid, approaching diffusion limits (14). Therefore, in a biological system such as a cell membrane, $HO^\bullet$ would react very close to its point of formation, which is typically a ligation site for redox-active iron. This is often referred to as "site-specific" reactivity (15, 16). In such systems, it is difficult to diagnose for $HO^\bullet$ intermediacy by using scavengers or traps because high enough concentrations for achieving localized competition are often beyond practical limits (16).

Superoxide per se can also oxidize biological substrates, but is rather selective in this. For example, O_2^- readily oxidizes iron–sulfur clusters and inactivates proteins bearing these groups (12), but unlike its conjugate acid, hydroperoxyl radical ($HOO^\bullet$), cannot trigger lipid peroxidation by abstracting allylic hydrogens (17). However, O_2^- reacts extremely rapidly with another naturally occurring (and also poorly oxidizing) radical, nitric oxide ($^\bullet NO$), to give peroxynitrite ($ONOO^-$)/peroxynitrous acid (ONOOH), a strong general oxidant and nitrating agent (18, 19). A variety of cells, including neutrophils, macrophages, neurons, and endothelial cells can produce both O_2^- and $^\bullet NO$ through the respective actions of various oxidases and nitric oxide synthases (20). The true oxidant in the $ONOO^-$/ONOOH system appears to be ONOOH, which decays rapidly via an activated intermediate that is diagnostically indistinguishable from $HO^\bullet$ (18, 19, 21). At pH 7.4 and 37°C, ~20% of the ONOOH/$ONOO^-$ pair exists as ONOOH, as its pK_a is ~6.8 (22). Peroxynitrite is known to be generated by activated macrophages, neutrophils, and endothelial cells, and it may play an important role in pathologies such as chronic inflammation, ischemia/reperfusion injury, and atherosclerosis (20, 23).

In addition to cellular sources, there are numerous environmental sources of ROS, important examples being ionizing and non-ionizing radiation. Of growing biomedical interest in the latter category are near ultraviolet radiation (UVA, 320–400 nm) and visible radiation (400–700 nm), which, together with appropriate photoexcitable compounds (sensitizers) and molecular oxygen, can produce oxidative injury through photodynamic action (24). UVA is insidious because *i*) it passes through the stratospheric ozone layer, unlike shorter wavelength radiation, and *ii*) it comprises a major part of the spectral output of commercial tanning lamps (25). Endogenous (intracellular) UVA-absorbing sensitizers include tetrapyrroles (e.g., protoporphyrin IX, bilirubin), flavins (FMN, FAD), and reduced pyridine nucleotides (NADH, NADPH), whereas exogenous sensitizers can be found in certain drugs, cosmetics, and food additives (25–28). Likewise, there are numerous sensitizers, both naturally occurring and synthetic, that are activated by visible light. Most prominent among these are the dyes/pigments being developed for therapeutic purposes, e.g. photodynamic therapy for in vivo or ex vivo eradication of tumors (29, 30). Singlet oxygen in the $^1\Delta_g$ state ($^1O_2$) has been reported to be an important ROS in UVA-driven reactions, particularly at longer wavelengths (31), and also in many reactions involving exogenous sensitizers and visible light (24, 32). Singlet oxygen, which is not a radical, is generated by ~22.5 kcal of energy transfer from a relatively long-lived sensitizer triplet (^3S) to ground state oxygen ($^3O_2$). Photodynamic reactions may also give rise to reduced oxygen species such as O_2^-, H_2O_2, and $HO^\bullet$; these frequently derive from hydrogen or electron transfer from a reductant (RH) like NAD(P)H or ascorbate to ^3S, followed by autoxidation of the latter (32). Reactions stemming from ^3S/RH encounters are referred to as Type I, whereas those stemming from $^3S/^3O_2$ encounters are referred to as Type II (33).

Ozone (O_3) is a bona fide ROS, but curiously is often omitted from discussions about ROS pathobiology. In the lower atmosphere of heavily populated cities, O_3 is a major pollutant generated by photochemical reactions between nitrogen oxides and trace hydrocarbons (14). Like $^1O_2$, O_3 is a powerful non-radical oxidant, which can produce free radicals in vitro and in vivo while provoking

membrane lipid peroxidation, enzyme inactivation, and other cytodamage (34–36). The primary target of O_3 toxicity is the lung, where respiratory bronchioles can undergo various types of structural and functional damage (37). Similar to many of the other ROS described, O_3 has been shown to induce cytoprotective antioxidant enzymes, both in cellular and animal model systems (38).

Lipid peroxidation and lipid hydroperoxide formation

Unsaturated phospholipids, glycolipids, and cholesterol in cell membranes and other organized systems are prominent targets of oxidant attack. This can result in lipid peroxidation, a degenerative process that perturbs structure/function of the target system, often with cytopathological consequences (1–5). Lipid peroxidation has been linked to a variety of disorders, including atherogenesis, ischemia–reperfusion injury, and UV-induced carcinogenesis (3). It may also play a role in the cytotoxic effects of oxidant-based chemotherapeutic and phototherapeutic drugs (4, 29). As shown in **Fig. 1**, involvement of reduced oxygen species in lipid peroxidation may commence with the reduction or dismutation of metabolically or photochemically generated O_2^{-} to H_2O_2. Fenton-type reduction of H_2O_2 by suitably chelated iron (e.g., membrane-associated Fe^{2+}) produces $HO^{\bullet}$, which can trigger chain peroxidation by abstracting allylic hydrogens from proximal unsaturated lipids (LHs). For phospholipids, these hydrogens would typically derive from *sn*-2 fatty acyl groups, whereas for cholesterol, the C-7 hydrogen is most reactive in this regard (39). Rapid addition of $^3O_2$ to the resulting lipid radicals ($L^{\bullet}$) propagates the reaction via peroxyl radical ($LOO^{\bullet}$) intermediates, with concomitant formation of lipid hydroperoxide (LOOH) species (1–4). Because of their increased polarity and long lifetimes compared with free radical precursors, LOOHs may be able to migrate from points of origin to more sensitive sites, e.g. sites at which iron-mediated cytotoxicity might be elicited (see below). Such movements (if detoxifying enzymes are not encountered) might be spontaneous or facilitated by lipid transfer proteins, possibilities that are currently under investigation in the author's laboratory.

In discussing free radical-mediated lipid peroxidation, it is important to point out that two homologous or heterologous peroxyl radicals have a finite possibility of undergoing Russell-type disproportionation to give an alcohol, a ketone, and oxygen (40). The oxygen is either $^1O_2$ or $^3O_2$, and the ketone is correspondingly in the ground state or triplet excited state. The luminescence associated with free radical peroxidation is often attributed to these reactions (41). It has been well established that lipid peroxidizability in model membranes and cells increases not only with polyunsaturated lipid content, but also with degree of fatty acyl unsaturation (42–44). With regard to the latter, spin trapping EPR (electron paramagnetic resonance) studies have shown that the rate of overall lipid peroxidation in oxidatively stressed leukemia cells increases exponentially with the number of *bis*-allylic hydrogens (44). This contrasts with the situation in homogeneous solution, where the peroxidation rate varies linearly with *bis*-allylic hydrogen content (45). This discrepancy is presumably related to the fact that most cellular lipids are closely packed and as such are more interactive in terms of chain propagation.

A similar overall mechanism would apply for ONOOH- or O_3-induced lipid peroxidation. In the case of ONOOH, homolytic decomposition gives $HO^{\bullet}$ and $O_2N^{\bullet}$, either of which could act as a H-abstracting initiator (46). According to a more recent proposal, however, the more probable initiator is an activated isomer of ONOOH with properties similar to $HO^{\bullet}$ (21). Surging interest in $^{\bullet}NO$-related

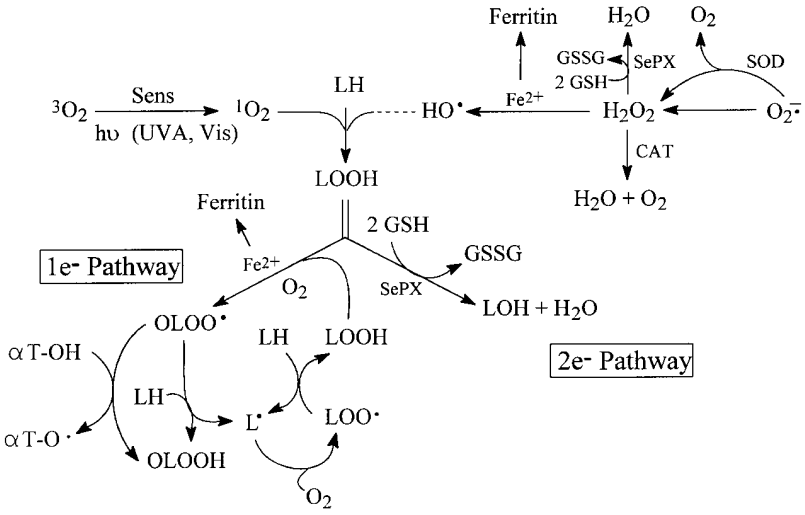


Fig. 1. Scheme depicting important routes of lipid hydroperoxide (LOOH) formation and turnover in oxidatively challenged cells. ROS such as $^1O_2$ generated by photodynamic action (Sens/hν) or $HO^{\bullet}$ generated by Fenton chemistry (H_2O_2/iron) gives rise to primary stage LOOHs. These LOOHs may undergo iron-mediated one-electron reduction and oxygenation to give epoxyallylic peroxyl radicals ($OLOO^{\bullet}$), which trigger exacerbating rounds of free radical-mediated lipid peroxidation. For $^1O_2$ systems, radical chemistry would start here, whereas for $HO^{\bullet}$ systems, radical chemistry would continue to be propagated. Alternatively, LOOHs may undergo two-electron reduction to redox-inert alcohols (LOHs); these reactions are typically catalyzed by GSH-dependent selenoperoxidase(s) (SePX), most prominently phospholipid hydroperoxide glutathione peroxidase (PHGPX). This represents a secondary (reparative) level of cytoprotection. Other antioxidant enzymes, including glutathione peroxidase (GPX, another SePX), catalase (CAT), and members of the superoxide dismutase (SOD) family protect mainly at the primary (preventative) level. Agents such as α-tocopherol (α-TOH) and ferritin can suppress LOOH formation by protecting at both the primary and secondary stages of lipid peroxidation.

cytotoxicity should lead to further clarification of this mechanism in the near future. Ozone can add across an unsaturated fatty acid double bond to give a 1,2,3-trioxolane, O–O homolysis of which gives an oxygen-centered diradical (47). The latter may undergo β-scission to split out a carbon-centered radical or rearrange to a carbonyl oxide, which in turn produces radicals via β-scission. As in HO•-triggered reactions (Fig. 1), these radicals can set peroxidation chains in motion, leading to formation of LOOHs and various other characteristic intermediates and by-products.

In contrast to HO• or ONOOH, $^1O_2$ can react directly with unsaturated fatty acyl groups or with cholesterol (see Fig. 1) to give LOOHs with double bonds shifted to the allylic position. This is an example of the "ene" reaction of $^1O_2$ with olefins (32). All atoms of the hydroperoxyl group derive from $^1O_2$ and the target lipid. This contrasts with a free radical-generated hydroperoxyl group, which derives from $^3O_2$ and another H-donating lipid. A unique attribute of $^1O_2$-generated LOOHs is that in the absence of reductants and metal catalysts, they accumulate linearly without lag as a function of $^1O_2$ generation (4). It is unfortunate that many articles and reviews continue to give the impression that all lipid peroxidation reactions are free radical in nature, notwithstanding the fact that $^1O_2$-mediated peroxidation does not per se involve free radicals. On the other hand, radical-mediated peroxidation might give rise to $^1O_2$ via LOO• disproportionation (see above), and this $^1O_2$ might produce new LOOHs, thereby contributing to overall peroxidative damage. However, evidence in support of such a mixed mechanism is rather limited thus far (48).

Enzymatic and non-enzymatic inhibitors of lipid peroxidation

Eukaryotic cells are equipped with a variety of primary and secondary defenses against lipid peroxidation and other deleterious effects of oxidative stress (49, 50). Both constitutive and inducible systems have been described (50). Potentially lethal injury can occur if these defenses are overwhelmed. Primary defenses are mainly preventative, whereas secondary defenses have a "back-up" protective role, which might typically involve excision/repair of any lesions that do develop. Primary cytoprotection relies on the scavenging/inactivation of ROS or redox metal ions before lipid peroxidation takes place. This aspect has been amply covered in numerous other reviews (3, 12, 49, 50), and, therefore, will be touched upon only briefly. Enzymes involved in primary cytoprotection include *i*) the copper/zinc-dependent (cytosolic) and manganese-dependent (mitochondrial) superoxide dismutases (SODs); *ii*) cytosolic and mitochondrial glutathione peroxidase (GPX), which scavenges H_2O_2 efficiently at relatively low concentrations (low K_M); and peroxisomal catalase (CAT), which scavenges H_2O_2 efficiently at relatively high concentrations (high K_M) (Fig. 1). (GPX may also be effective at the secondary stage, e.g., by detoxifying fatty acid hydroperoxides; see below.) On the other hand, there are no known specific enzymatic scavengers for $^1O_2$ or O_3; however, like HO•, these species can be intercepted (often indiscriminately) by a variety of low molecular weight antioxidant compounds (51). Chain-breaking antioxidants such as α-tocopherol (α-TOH) and butylated hydroxytoluene (BHT) can afford primary as well as secondary stage protection; by competing with LHs for peroxyl radicals (Fig. 1), they also produce LOOHs, but in much lower overall yield than without competition, and this basically accounts for their efficacy. Provocative recent studies have revealed that •NO can also function as a chain-breaking antioxidant by reacting with peroxyl radicals, and appears kinetically to be more effective in this than α-TOH, at least in model systems (52, 53). In the process, lipid nitrites and nitrates appear to be generated via rearrangement of unstable nitrosoperoxyl intermediates (53). Thus, •NO by itself can act as a lipid antioxidant, but upon reacting with O_2^- it becomes $ONOO^-$/ONOOH, a powerful lipid prooxidant (see above). The expression and regulation of this dual activity in vivo, e.g., in the vascular system, is clearly a matter of great importance (23). In addition to these various agents there are iron-sequestering proteins, lactoferrin and ferritin being prime examples (3), which play an important role in limiting peroxidation potency, both at the primary and secondary levels (Fig. 1).

Lipid hydroperoxides as mechanistic reporters in oxidant systems

Lipid peroxidation and other oxidative damage can be "diagnosed" for free radical or $^1O_2$ involvement in a variety of ways, each with certain advantages and disadvantages. One popular approach is to determine whether a reaction is inhibited by well-established free radical or non-radical scavengers, i.e., compounds that intercept these species at high rates. Along these lines mannitol has been used for HO•, butylated hydroxytoluene (BHT) for LOO• or LO•, and azide or histidine for $^1O_2$ (14). Unfortunately, many of these agents lack absolute specificity. For example, azide can intercept HO• as well as $^1O_2$, and histidine might chelate and thereby dislocate metal ions involved in site-specific HO• formation. Moreover, highly polar scavengers like azide and mannitol interact poorly with membranes, whereas low polarity scavengers like BHT may interact well, but in doing so perturb membrane structure, possibly interfering with chain propagation for this reason. Approaches based on stimulation of a reaction have also been used. For example, substituting D_2O for H_2O increases $^1O_2$ lifetime by about 15-fold and on this basis D_2O would be expected to stimulate $^1O_2$-mediated, but not radical-mediated reactions (54). However, oxidizable membrane lipids are typically located in H_2O-poor environments, and this might preclude a D_2O effect. Thus, not seeing such an effect in a peroxidation system would not necessarily rule out $^1O_2$ intermediacy (55).

A more rigorous alternative to these approaches is based on product screening, i.e., identification of species that are uniquely characteristic of either $^1O_2$ or free radical intermediacy. The lipid substrate that has been exploited to greatest advantage in this regard is cholesterol. Although phospholipid polyunsaturated fatty acyl (PUFA)

groups have also been considered as possible reaction probes, cholesterol is clearly superior because *i*) unlike PUFAs in natural membranes, it exists as a single molecular species, making product isolation and characterization much less complicated; *ii*) its oxidation products are ready for analysis without the need for potentially artifactual hydrolysis steps; and *iii*) unlike phospholipids, it can be readily transfer-radiolabeled in membranes and cells without transfer protein requirement. Cholesterol products generated by ionizing radiation, photodynamic action, ozone exposure, and other oxidant conditions have been well characterized in simple systems such as organic solvents, micelles, and liposomal membranes (39, 56–62). In free radical-mediated reactions, the epimeric pair 7α-OOH and 7β-OOH (**Fig. 2**) are generally the most prominent hydroperoxide products, with lesser amounts of the dihydroxy derivatives (7α-OH and 7β-OH), 7-ketone (7-one), epimeric 5,6-epoxides, and various other species (39, 58, 61). The free radical-derived hydroperoxides arise via direct attack of an oxidant on cholesterol (initiation) and/or via propagation reactions (see Fig. 1). In $^1O_2$-mediated reactions, three characteristic peroxides are seen: 5α-OOH, 6α-OOH, and 6β-OOH (Fig. 2), the first of these being generated at the highest rate in most reaction systems (56, 57, 59, 60, 62). Although $^1O_2$ does not produce 7α- or 7β-OOH, this pair can arise via allylic rearrangement of 5α-OOH, which is especially pronounced in low polarity solvents (63). On the other hand, no 5α-OOH is generated in reactions that are purely free radical in nature (58). Like other monoenoic lipids, cholesterol is oxidized at a relatively low rate compared with PUFA-containing phospholipids. However, problems of detection and quantitation have been alleviated for the most part by the availability of labeled cholesterol of high specific radioactivity and also by the development of ultrasensitive new detectors for chromatographic analysis (64–66).

High performance analytical techniques

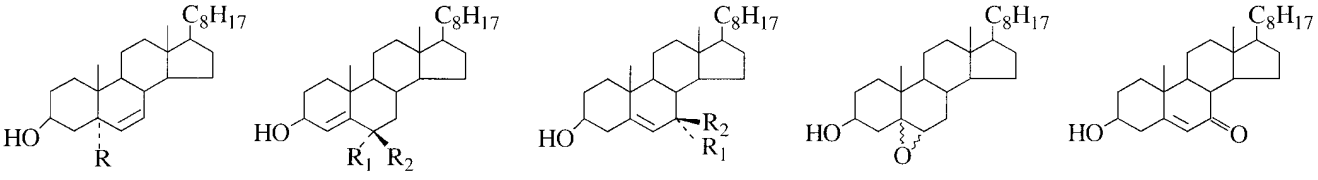
A broad array of techniques is available for measuring lipid peroxidation and LOOHs, ranging from relatively simple "bulk" methods such as the thiobarbituric acid assay to sophisticated HPLC-based approaches with extremely high sensitivity and specificity. Two of the latter have been used specifically for LOOH separation/detection, one using chemiluminescence detection, HPLC-CL (67, 68), and the other mercury cathode electrochemical detection, HPLC-EC(Hg) (69–71). Both of these approaches represent state-of-the-art in LOOH analysis, although HPLC-EC(Hg), which was developed more recently in this laboratory (65), has distinct advantages and may be the method of choice for future work in this area. Most of the studies to be described dealing with biological LOOH reduction involved the use of HPLC-EC(Hg) for peroxide analysis.

LIPID HYDROPEROXIDE TURNOVER IN BIOLOGICAL SYSTEMS

Toxic effects of one-electron reduction

In addition to perturbing membranes directly, LOOHs arising from free radical- or $^1O_2$-mediated lipid peroxidation can undergo one-electron reduction to oxyl radical ($LO^\bullet$) intermediates. This reaction, which is kinetically more favorable than one-electron oxidation (72), is typically mediated by Fe^{2+}, which would necessarily be chelated in close proximity to a reactive LOOH, e.g., on a cell membrane. At least three possibilities are open to $LO^\bullet$ radicals: *i*) direct H-abstraction and initiation of chain peroxidation; *ii*) β-scission with formation of aldehydes and alkyl radicals; and *iii*) rearrangement and oxygenation to give epoxyallylic peroxyl radicals ($OLOO^\bullet$). Recent studies with model systems have shown that the latter alternative is far more favorable than the others (73, 74). In that case, $OLOO^\bullet$ (rather than $LO^\bullet$, as widely assumed) would trigger rounds of free radical-mediated lipid peroxidation, during which new hydroperoxide substrates are generated (Fig. 1). Reactions of this type would exacerbate the damaging effects of primary peroxidation alone (4, 5).

Metal ion-catalyzed oxidation of low density lipoprotein



	R
5α-OOH	OOH
5α-OH	OH

	R_1	R_2
6α-OOH	OOH	H
6β-OOH	H	OOH
6α-OH	OH	H
6β-OH	H	OH

	R_1	R_2
7α-OOH	OOH	H
7β-OOH	H	OOH
7α-OH	OH	H
7β-OH	H	OH

5,6-epoxide

7-one

Fig. 2. Structural formulas of relevant cholesterol oxidation products. The species shown are as follows: 3β-hydroxy-5α-cholest-6-ene-5-hydroperoxide (5α-OOH); 5α-cholest-6-ene-3β,5-diol (5α-OH); 3β-hydroxycholest-4-ene-6α-hydroperoxide (6α-OOH); 3β-hydroxycholest-4-ene-6β-hydroperoxide (6β-OOH); cholest-4-ene-3β,6α-diol (6α-OH); cholest-4-ene-3β,6β-diol (6β-OH); 3β-hydroxycholest-5-ene-7α-hydroperoxide (7α-OOH); 3β-hydroxycholest-5-ene-7β-hydroperoxide (7β-OOH); cholest-5-ene-3β,7α-diol (7α-OH); cholest-5-ene-3β,7β-diol (7β-OH); 5,6-epoxide: unspecified mixture of 5,6α-epoxy-5α- and 5,6β-epoxy-5β-cholestan-3β-ol.

(LDL) is an important example of damaging lipid peroxidation that is driven by one-electron LOOH turnover. Among other things, this process is associated with the accumulation of phospholipid-, cholesterol-, and cholesteryl ester-derived hydroperoxides, along with aldehyde degradation products (75–77). Various lines of evidence suggest that LDL oxidation is an early vascular event that contributes to the development of atherosclerosis (78). The free radical-mediated lipid peroxidation associated with LDL oxidation causes apoB-100 protein modification, which in turn greatly increases LDL uptake by vascular macrophages, leading ultimately to the formation of atherosclerotic plaques (75, 79). In addition to this, oxidized LDL has chemotactic and cytotoxic properties (78) and can elicit stress signaling (80, 81). Most germane to this discussion is the question of peroxidative mechanism and the prospect that preexisting LOOHs in LDL play a crucial role in this mechanism. Carefully prepared human LDL has been shown to contain trace amounts of cholesteryl ester hydroperoxides (CEOOHs) by HPLC-EC(Hg) analysis, e.g., 6–12 pmol/mg protein, corresponding to ~1 CEOOH for every 200–400 particles (82). These and other even less abundant LOOHs were reduced to undetectable levels by treating with GSH and the selenoperoxidase PHGPX (see below). This material was found to be much more resistant to a Cu^{2+}-induced peroxidative burst than an untreated control, whereas material enriched in CEOOH by aging or photogeneration in situ was less resistant (82). These findings clearly demonstrate that at any given antioxidant level, LDL oxidizability depends strongly on its starting LOOH content, which in vivo may be governed by contact with lipoxygenases, lecithin:cholesterol acyltransferase, or LOOH-bearing cells (78, 82, 83). By undergoing one-electron redox chemistry, these LOOHs would drive peroxidative chain reactions (Fig. 1), thereby promoting oxidized LDL's pathophysiological potential.

One-electron reduction of primary LOOHs may also occur during $^1O_2$-mediated photodynamic reactions (Fig. 1). This reduction would be light-independent and governed by many different factors, including the availability of iron, electron donors, and antioxidants. The interrelationship of these factors in complex systems is still poorly defined. In addressing this question for dye/light ($^1O_2$)-treated leukemia cells (84), we recently showed, using HPLC-EC(Hg) for peroxide analysis, that 5α-OOH and 6α,6β-OOH ($^1O_2$ adducts) were dominant in the early stages of photooxidation, whereas 7α,7β-OOH (radical products) became so after prolonged irradiation or during dark incubation after a dye/light dose. Using the 7-OOH/5α-OOH ratio as a sensitive, internally consistent index for monitoring these changes, we found that this ratio increased steadily in the dark after a dye/light dose, but that treating cells with DFO prevented this. The rise in 7-OOH/5α-OOH was greatly enhanced by lipophilic iron chelates, e.g., ferric-8-hydroxyquinoline [$Fe(HQ)_2$], but diminished by butylated hydroxytoluene (BHT) (**Fig. 3A**). Concomitantly, post-irradiation BHT was able to "rescue" cells from progressively greater killing, presumably by intercepting lipid-derived radicals generated by primary

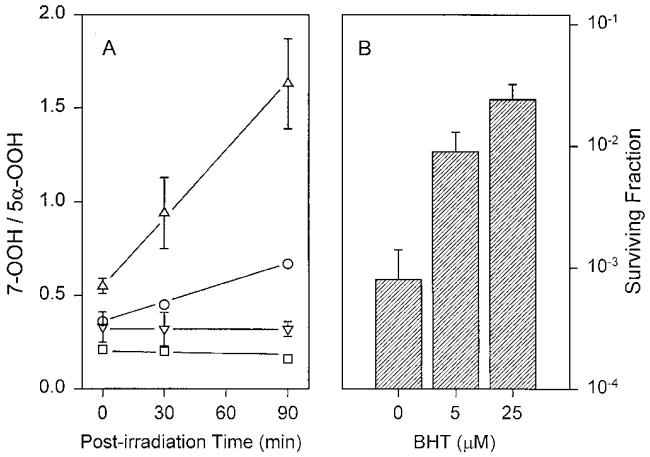


Fig. 3. Effect of butylated hydroxytoluene on post-irradiation changes in cholesterol hydroperoxide ratio and cell killing. L1210 cells were preincubated without (control) or with 0.5 μm $Fe(HQ)_2$, and then exposed to a 0.38 J/cm^2 light fluence in the presence of 10 μm merocyanine 540, a sensitizing dye. (A) Cholesterol hydroperoxide analysis. Immediately after irradiation, BHT in ethanol or ethanol alone was added to the control and $Fe(HQ)_2$-treated cells; concentrations of BHT and ethanol in cell suspension were 25 μm and 0.5%, respectively. During subsequent dark incubation at 37°C, samples were extracted and recovered lipid fractions were analyzed by HPLC-EC(Hg). Temporal changes in 7-OOH/5α-OOH ratio are plotted: (○) Control + ethanol; (□) Control + BHT/ethanol; (△) $Fe(HQ)_2$ + ethanol; (▽) $Fe(HQ)_2$ + BHT/ethanol. (B) Photokilling. Immediately after irradiation, non-$Fe(HQ)_2$-treated cells were treated with BHT at the indicated concentrations or with ethanol vehicle (0 μm BHT). After these additions, the cells were incubated in the dark for 20 h, after which survival was assessed by clonal assay. Values in (A) and (B) are means ± deviation of measurements from duplicate experiments. From Geiger et al. (84), with permission.

LOOH degradation (Fig. 3B). These findings, like those described above for LDL, clearly illustrate that biological LOOHs are susceptible to iron-catalyzed one-electron turnover. By triggering chain reactions, this can expand peroxidative damage and along with it elicit a variety of responses ranging from antioxidant enzyme induction to cell death (see below).

Detoxification via two-electron reduction

As already mentioned, a second line of enzymatic defense against peroxidation injury exists in most eukaryotic cells. In general, this involves removal of LOOHs at damage sites, followed by repair reactions. For some reactive species, which lack primary enzymatic scavengers, this appears to be the only means of coping at the metabolic level. Singlet oxygen is one case in point; ozone is another. Unlike excision/repair of thymine dimers in DNA, excision/repair of LOOH lesions in cell membranes has not been well characterized in mechanistic terms. Three intracellular enzymes with peroxidatic activity have been implicated in LOOH detoxification: glutathione-peroxidase (GPX); phospholipid hydroperoxide glutathione peroxidase (PHGPX), and non-seleno GSH-S-transferase type α (GSTα) (85, 86). The SePXs have been studied far more extensively and are considered to be more important in

overall LOOH disposal. In support of this are findings that Se-deficient cells accumulate LOOHs more rapidly under oxidative challenge than normal counterparts and die off faster (87, 88). GPX and PHGPX both contain an active site selenocysteine which participates in the two-electron reduction of peroxides to alcohols (Fig. 1). However, the enzymes differ in functional size (GPX being an 85 kDa tetramer and PHGPX a 20 kDa monomer), subcellular distribution, and amino acid sequence (only ~30% homology) (89). They also exhibit striking differences in peroxide reactivity. PHGPX can act directly on phospholipid hydroperoxides (PLOOHs) in membranes (86, 90), whereas GPX is unreactive unless *sn*-2 fatty acyl bonds are cleaved to liberate fatty acid hydroperoxides (91, 92). For membrane PLOOHs, elimination could involve *i*) sequential action of peroxide/Ca^{2+}-stimulated phospholipase A_2 (PLA_2) and GPX (92) or *ii*) sequential action of PHGPX and PLA_2 (90), followed in either case by reacylation of the resulting lysolipids (**Fig. 4**). The latter process might involve reinsertion of the same fatty acyl group or remodeling of some type. The biological importance of the GPX pathway (excision/reduction/repair) relative to the PHGPX pathway (reduction/excision/repair) is not yet certain, although evidence in favor of the latter is accumulating rapidly. For example, by use of kinetic modeling (93), it has been estimated that the reductive flux of PLOOHs through mitochondrial PHGPX is at least 10^4-fold greater than the hydrolytic flux through PLA_2, suggesting that PHGPX is far more efficient than GPX in removing PLOOHs. In another study (94), treatment of hepatoma cells with a [^{14}C]PLOOH resulted in Se-stimulated accumulation of [^{14}C]PLOH, but not hydroperoxy- or hydroxy-fatty acid, suggesting that reductive detoxification was mediated by PHGPX rather than GPX or GSTα. Of added interest are recent studies showing that cells transfected with a PHGPX expression system were better equipped to detoxify LOOHs and survive peroxidative stress than normal counterparts (95, 96). There is a growing consensus based on these and related findings that under physiological conditions, GPX acts mainly on substrates of relatively high polarity, e.g., H_2O_2 and fatty acid hydroperoxides. In contrast, PHGPX, the more versatile of the two enzymes, acts not only on H_2O_2 (albeit less efficiently than GPX), but also on a broad range of lower polarity substrates, including phospholipid, cholesterol, and cholesteryl ester hydroperoxides (86, 97, 98).

Cholesterol comprises 40–45 mol % of eukaryotic plasma membrane lipid; therefore, how cells deal with cholesterol-

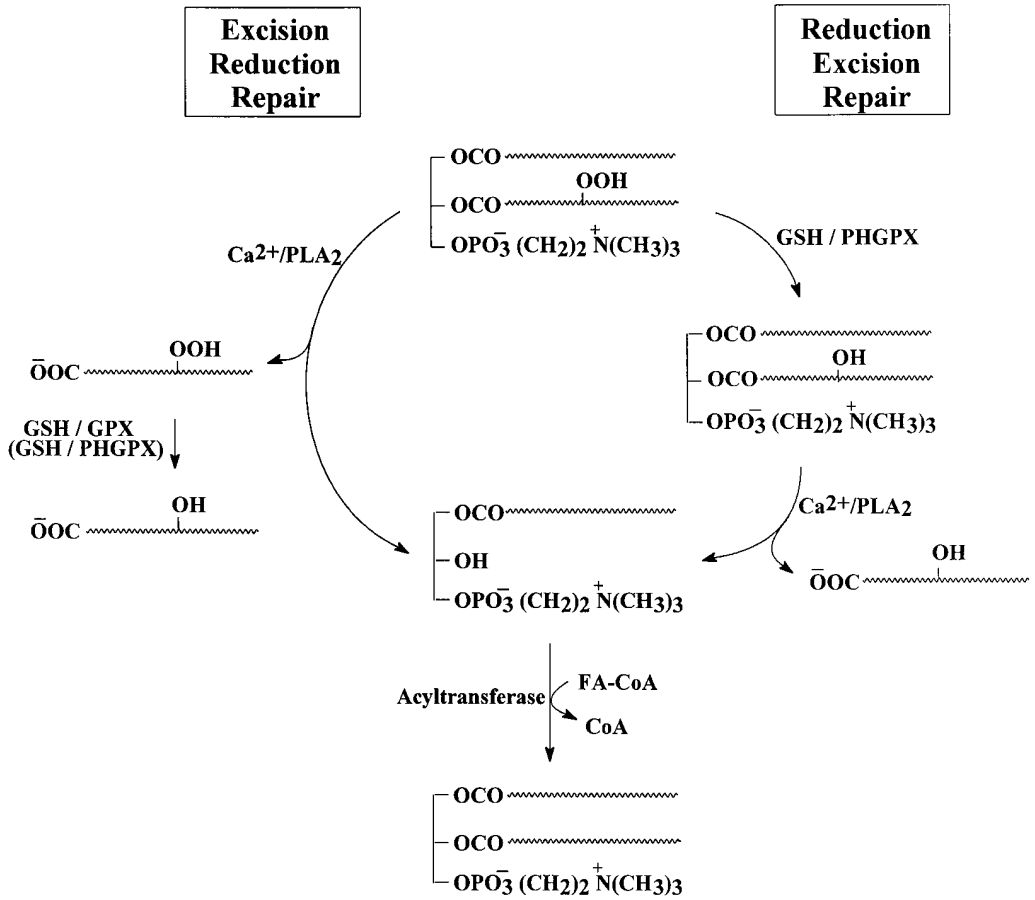


Fig. 4. Proposed pathways of cytoprotection against the deleterious effects of phospholipid hydroperoxides. The Excision/Reduction/Repair pathway involves consecutive action of phospholipase A_2, glutathione peroxidase, and an acyltransferase; whereas the Reduction/Excision/Repair pathway involves consecutive action of phospholipid hydroperoxide glutathione peroxidase, phospholipase A_2, and an acyltransferase.

derived hydroperoxides (ChOOHs) is also important. ChOOHs are completely resistant to GPX (99), ruling out any involvement of PLA_2/GPX in the cytoprotection of ChOOH-challenged cells. The first clues as to how ChOOHs are detoxified came from experiments showing that LOOH-containing erythrocyte ghosts were only partially protected against Fe^{2+}-induced peroxidation by PLA_2/GSH/GPX treatment, but completely protected by GSH/PHGPX treatment (98, 99). This provided the first evidence that PHGPX can react with membrane ChOOHs in addition to PLOOHs. Recent studies have shown that several photochemically generated ChOOHs are reduced at different rates by the GSH/PHGPX system (100). Rate constants for peroxide disappearance in several different model systems (Triton X-100 micelles, unilamellar liposomes, and red cell ghosts) were found to increase in the following order: 5α-OOH < 6α-OOH = 7α,7β-OOH < 6β-OOH. Under the conditions described (100), the 6β-OOH/5α-OOH rate ratio varied between 4 and 8 for the different reaction systems. Loss of each ChOOH, as assessed by HPLC-EC(Hg), was accounted for by formation of the expected diol (ChOH) product, as assessed by TLC with ^{14}C-radioimaging. A similar kinetic profile was observed when ChOOH mixtures were incubated in the presence of GSH and Triton lysates of selenium-replete [Se(+)] L1210 cells, implying PHGPX involvement (**Fig. 5**). In keeping with this, there was little detectable reaction when GSH was omitted from the reaction mixture or when lysates from selenium-deficient [Se(−)] cells were used; Se(−) cells were ~50-fold depressed in PHGPX activity. Experiments with [^{14}C]ChOOHs showed that 5α-OOH and 6β-OOH in liposomal form are taken up at equal rates by Se(+) cells, yet the former is much more cytotoxic, its concentration for 50% lethality being about one-fifth that of 6β-OOH (100). The logical implication is that 5α-OOH has a long lifetime in cells due to its relatively slow PHGPX-mediated metabolism, and this accounts for its greater cytotoxicity. The latter presumably reflects the ability of 5α-OOH, like other LOOHs, to undergo iron-catalyzed one-electron reduction in cells (Fig. 1), similar to that demonstrated for 5α-OOH-containing model membranes (101). The strong cytotoxic potential of 5α-OOH due to inefficient catabolism becomes even more important when its known high rate of $^1O_2$-mediated generation is taken into account (66). Therefore, under conditions of photodynamic stress, 5α-OOH would be the most damaging ChOOH, and possibly one of the most damaging of all LOOHs generated in any given system.

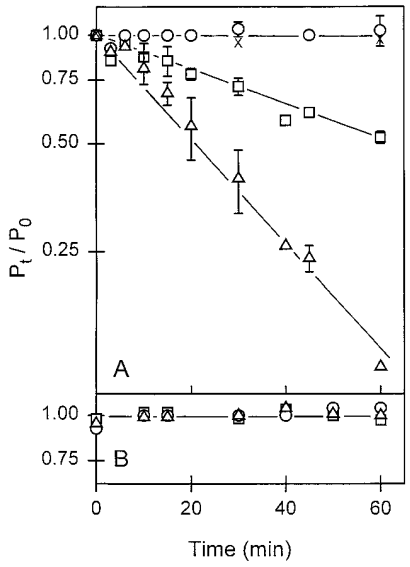


Fig. 5. Time courses for the reduction of cholesterol hydroperoxides in the presence of GSH and lysates of selenium-replete (A) or selenium-deficient (B) L1210 cells. Reaction mixtures contained 40 μm each of 5α-OOH, 6β-OOH and 7α,7β-OOH, 5 mm GSH, 50 μm DFO, 100 μm EDTA, and cell lysate corresponding to ~2.3×10^7 cells/ml in 0.1% Triton X-100/PBS. Samples removed during incubation at 37°C were extracted and recovered ChOOHs were analyzed by HPLC-EC(Hg): (○) 5α-OOH; (△) 6β-OOH; (□) 7α,7β-OOH. A control system lacking GSH or PHGPX was also monitored: (x) total ChOOH. P_o and P_t denote peroxide concentrations at zero time and time *t*, respectively. Mean values from duplicate experiments are shown.

Although most of the current thinking about PHGPX centers on its antioxidant capabilities, there is also speculation (90) that the enzyme might be involved in the modulation of natural biological activities that are peroxide-dependent. For example, cyclooxygenase requires a low level of LOOH for optimal activity, an example of 'peroxide tone' (102). LOOH reduction by PHGPX would tend to deactivate cyclooxygenase and thereby down-regulate eicosanoid metabolism and associated physiological events. Similar reasoning may apply to PLA_2, protein kinase C, and other peroxide-responsive enzymes (see below). PHGPX's relative importance as a peroxide "regulator" versus peroxide "detoxifyer" may depend on the type of tissue involved, but this remains to be investigated.

Other aspects of LOOH catabolism besides GPX- or PHGPX-catalyzed reduction have been described. For example, lecithin:cholesterol acyltransferase (LCAT) associated with high density lipoprotein (HDL) has been shown to mediate the formation of CEOOH from PLOOH and cholesterol (83). As PLOOH is undetectable in plasma or after being added to plasma samples in molar excess over GSH (83), LCAT may complement PLA_2/GPX in ridding plasma of PLOOH. HDL-CEOOH formed in the process can be taken up by liver cells, where it undergoes reductive detoxification (103). The LCAT reaction could be especially important under high stress conditions, i.e., when plasma GPX capacity is exceeded (83). This may also apply for a recently reported non-SePX CEOOH reducing activity associated with both LDL and HDL (104).

INVOLVEMENT OF LIPID HYDROPEROXIDES IN OXIDATIVE STRESS SIGNALING

Survival pathways versus death pathways

According to Fig. 1, serious peroxidative damage can be averted if two-electron detoxification of strategic primary LOOHs (e.g., $^1O_2$ adducts) overtakes one-electron toxicity enhancement. On the other hand, if LOOHs accumulate

at a high rate and iron-catalyzed reduction takes hold, then detoxification is overwhelmed and potentially lethal chain peroxidation may be unleashed. The determinants of how different LOOHs will partition between the one-electron and two-electron pathways in an actual cell setting are still poorly defined. An area of related interest that is attracting considerable attention is oxidative stress-induced cell signaling, e.g., signaling that may originate with lipid peroxidation and culminate with apoptotic (genetically programmed) cell death (6, 105). Apoptosis is characterized by cell shrinkage, loss of plasma membrane asymmetry, protease and endonuclease activation, and internucleosomal fragmentation of nuclear DNA (106). According to recent findings (107, 108), apoptosis is mediated by mitochondrial events rather than nuclear events, DNA fragmentation, for example, appearing to be an epiphenomenon. In contrast to apoptosis, necrosis (non-programmed death) is characterized by cell swelling, lysis, and random DNA fragmentation. Whereas necrotic cells can elicit inflammation in vivo, apoptotic cells are non-inflammatory, typically being removed by phagocytosis (109). Apoptosis has been induced by oxidizing conditions such as H_2O_2 treatment (110), peroxynitrite treatment (111), ionizing radiation (112), UVA radiation (113), and dye/visible light treatment (114, 115). Involvement of lipid peroxidation has been inferred in several instances by showing, for example, that ordered DNA fragmentation is suppressed by the SePX mimetic ebselen (114), or by Trolox and other phenolic antioxidants (110, 111). Some of these agents have been used at very high concentration (up to 10 mm) and consequently their implied specificity as free radical interceptors could be open to question. Notwithstanding such uncertainties, the overall evidence presented thus far, albeit limited (110–116), argues quite persuasively for some early involvement of lipid peroxidation in oxidative stress-induced apoptosis. In seeking additional supporting evidence for this, we showed recently (W. Korytowski, P. G. Geiger, and A. W. Girotti, unpublished observations) that exogenous or endogenous peroxides can trigger an apoptotic cascade in leukemia cells (as evidenced by DNA laddering) and that this can be modulated by SePX enzyme(s). For example, Se(−) HL-60 cells were found to be more susceptible to t-butyl hydroperoxide-induced DNA fragmentation than Se(+) controls and this was accompanied by greater accumulation of LOOHs and loss of viability. All effects, including the DNA effects, were inhibited by ebselen and by BHT, implying that free radical-mediated lipid peroxidation triggered by t-butyl hydroperoxide was involved (88). Similar Se(−) versus Se(+) effects were observed when cells were challenged photodynamically, suggesting that in this case $^1O_2$-derived LOOHs were involved in apoptotic signaling. The exact nature of this involvement remains to be elucidated.

It is important to consider the extent of peroxidative injury when asking whether a stressed cell will either survive or succumb via apoptosis or necrosis. This can be viewed according to a pattern of graded responses such as the following: *i*) no net damage when constitutive antioxidant capacity is sufficient to either prevent or repair peroxide lesions; *ii*) relatively mild (sublethal) oxidative injury, which may trigger cytoprotective responses, e.g., induction of GSH and/or antioxidant proteins such as GPX, catalase, heme oxygenase, or ferritin (117–122); *iii*) more extensive damage, which triggers apoptosis because some undefined threshold is crossed, beyond which both constitutive and inducible repair capacity is exceeded; and *iv*) gross damage, which preempts any type of programmed metabolic response and results in necrosis. A scheme illustrating these different possibilities for lipid peroxidation-mediated stress is shown in **Fig. 6**. It should be emphasized that this scheme is hypothetical and the trends shown may not necessarily apply for all peroxide-challenged cells. There have been numerous reports (117–122) that mammalian cells can overproduce antioxidants such as GSH, GPX, CAT, and SODs in response to sublethal or modestly lethal levels of oxidant pressure (see category *ii* above). In some instances, a specific role of lipid peroxidation in signal transduction has been inferred based on various lines of evidence. Induction of heme oxygenase-1 (HO-1) and ferritin in UVA-exposed skin fibroblasts is an important example (121–123). Overexpression of these proteins is believed to be a defensive strategy against heme- and iron-mediated oxidative injury triggered by UVA-derived $^1O_2$ (31). Exposing fibroblasts to UVA also provokes iron-catalyzed lipid peroxidation, as indicated by formation of TBARS, 4-hydroxynonenal (4-HNE), and LOOHs. Recent studies have shown that lipid metabolites such as arachidonate and diacylglycerol (possibly upregulated because of peroxidation-activation of phospholipases C and A_2; see below) can activate HO-1 expression (123). This implies that protein kinase C (see below) and eicosanoid(s) are involved in the activation process. The peroxidation by-product 4-HNE was also shown to be a strong activator. Thus, there appear to be several different lipid-derived mediators of stress signaling in this system, each associated with peroxidative UVA damage. Interestingly, subcellular membranes were found to be more important in signal transduction than the plasma membrane, evidently because the former are in closer proximity to endogenous UVA-excitable sensitizers.

Phospholipase and protein kinase involvement

Activation of certain protein kinases and phospholipases is believed to play an important role in oxidant-induced signaling that stimulates gene expression associated with cytoprotection, apoptosis, or other responses (see Fig. 6). Highly noteworthy among these enzyme transducers are protein kinase C (PKC), mitogen-activated protein kinases (MAPKs) and hydrolases such as phospholipase C and Ca^{2+}-activated phospholipase A_2 (6, 124–126). There is a growing awareness that oxidant-derived species such as LOOHs are early intermediates in these pathways which might mimic the effects of certain growth factors, cytokines (e.g., tumor necrosis factor-α), or other natural agonists. This has stimulated great interest in oxidant derivatives as possible second messengers (6). Considerable attention has been directed to oxidant activation of PLA_2,

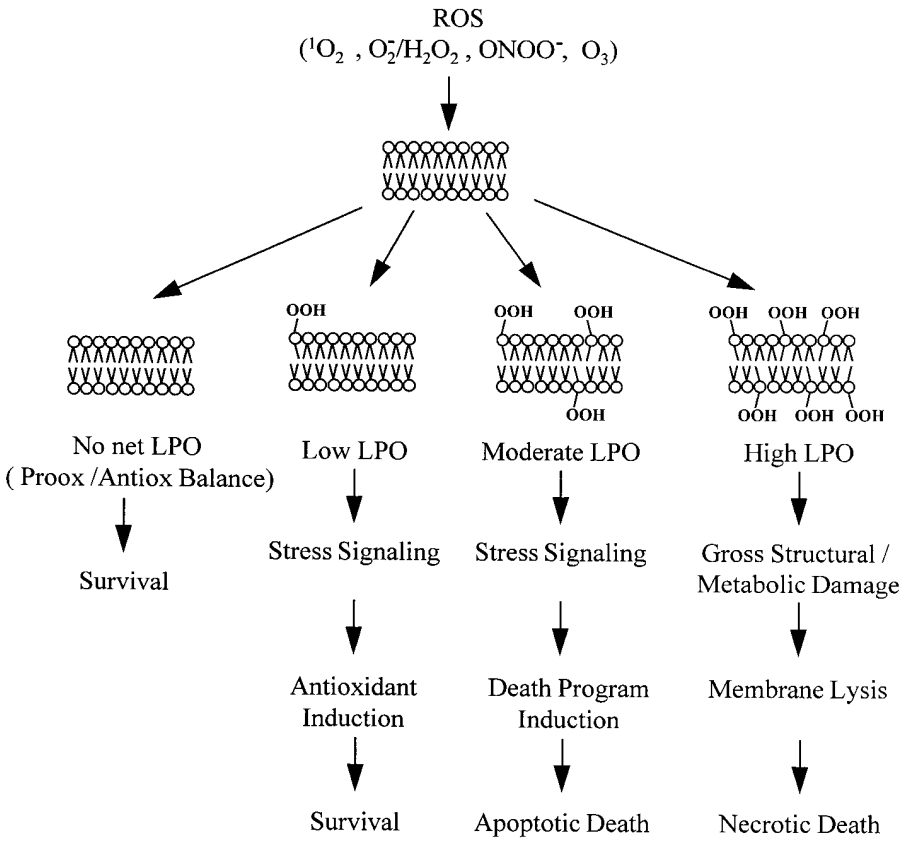


Fig. 6. Scheme showing possible levels of lipid peroxidation (LPO) involvement in ROS-induced signal transduction. Responses may range from *i*) no net effect (prooxidant-antioxidant standoff); *ii*) antioxidant enzyme induction at relatively low LPO pressure; *iii*) apoptotic death at moderate LPO pressure; and *iv*) necrotic death at high LPO pressure. Transition from one phase to another (e.g. ii to iii) may occur as some (undefined) threshold of damage is exceeded.

which, by releasing arachidonate from phospholipids, plays a crucial role in the production of eicosanoid lipid mediators such as prostaglandins, thromboxanes and leukotrienes. PLA_2 also gives rise to lysophospholipids, which may either exert direct effects or be metabolized to platelet activating factor (PAF) and other mediators. Phospholipid membrane studies carried out with secreted PLA_2 ($sPLA_2$, ~14 kDa) (126) and more recently with the cytosolic enzyme ($cPLA_2$, ~85 kDa) (124, 125) have clearly demonstrated that hydrolytic activity increases as a function of membrane PLOOH content. This effect has been observed with modestly peroxidized liposomes, as well as liposomes constituted with low levels (<15 mol %) of PLOOH, and is ascribed to alterations in phospholipid packing, along with greater Ca^{2+} binding. Importantly, the hydrolysis of PLOOHs greatly exceeds that of unoxidized lipids, and there is a more rapid liberation of oxidized fatty acids. (This was alluded to earlier in the context of PLOOH detoxification; see Fig. 4.) A second activation mechanism applies specifically to $cPLA_2$ (which acts preferentially on *sn*-2 arachidonyl groups), and involves PKC-catalyzed phosphorylation and translocation to the membrane (125). In this mechanism, PKC itself appears to be activated by membrane PLOOHs, and this in turn promotes $cPLA_2$ phosphorylation/activation, possibly via MAPK activation in cells (6, 125). In contrast to the first mechanism, this one shows much lower Ca^{2+} dependency and therefore may be operative at relatively low stress/peroxide levels, i.e., before intracellular [Ca^{2+}] is substantially elevated (6).

Simulation of natural signal transduction

As alluded to above, the ability of LOOHs and other intermediates/products of chain peroxidation to mimic some of the effects of natural agonists is attracting considerable interest. Platelet activating factor is an important example. Recent studies have shown that oxidatively modified (e.g., Cu^{2+}-treated) LDL contains HPLC-separable phospholipid derivatives that resemble PAF in biological activity, e.g., stimulation of neutrophil adhesion and smooth muscle cell proliferation (127). Moreover, low-activity cells could be made strongly responsive to both oxidized LDL lipid and PAF by transfecting with the PAF receptor. All of these effects could be strongly inhibited by PAF acetylhydrolase, implying that there was structural similarity between the phospholipid mimic and PAF itself. Although not yet defined structurally, the mimic is believed to be a mixture of oxidatively fragmented phospholipids whose *sn*-2 chains (some bearing aldehyde groups) are relatively short as in PAF. Fragmentation is attributed to β-scission

reactions that commonly occur during radical-mediated lipid peroxidation (73). These findings raise the possibility that PAF-like species in peroxidized LDL may contribute to its atherogenic potential, either by derivatizing apoB-100 protein to a form recognized by scavenger receptor or by activating leukocytes and stimulating smooth muscle cell growth (78, 79). Other aspects of related interest are expected to emerge as the pathologic consequences of LOOH-mediated lipid peroxidation continue to be explored.

SUMMARY AND PERSPECTIVES

Research on lipid peroxidation has intensified in recent years largely because of increasing awareness that this process may play an important role in UV-induced skin cancer, atherosclerosis, neurodegeneration, and various other disorders. Lipid hydroperoxides are well known intermediates of peroxidative reactions which are generally more long-lived than any free radical precursors, making intermembrane translocation within a cell, between cells, or between lipoproteins and cells possible.

As a result, LOOH toxicity and effector action could conceivably be manifested far beyond the site of LOOH origin, but relatively little is known about this. Similarly, much more needs to be learned about the factors that influence the reductive fate of LOOHs, i.e., toxicity enhancing one-electron reduction on the one hand versus detoxifying two-electron reduction on the other. In the case of one-electron reduction, these factors include availability of redox iron and electron donors, whereas for two-electron reduction, accessibility of LOOHs to GSH and PHGPX would be highly important. An additional exciting aspect which is just beginning to unfold relates to the role of LOOHs in signal transduction. Such signaling may determine whether a cell survives or succumbs to an oxidative insult. Progress in each of these complex areas is clearly important because of the far-reaching physiological and biomedical implications.

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