



## Lipid Peroxidation and Its Toxicological Implications

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Lipid peroxidation is a free radical oxidation of polyunsaturated fatty acids such as linoleic acid or arachidonic acid. This process has been related with various pathologies and disease status mainly because of the oxidation products formed during the process. The oxidation products include reactive aldehydes such as malondialdehyde and 4-hydroxynonenal. These reactive aldehydes can form adducts with DNAs and proteins, leading to the alterations in their functions to cause various diseases. This review will provide a short summary on the implication of lipid peroxidation on cancer, atherosclerosis, and neurodegeneration as well as chemical and biochemical mechanisms by which these adducts affect the pathological conditions. In addition, select examples will be presented where antioxidants were used to counteract oxidative damage caused by lipid peroxidation. At the end, isoprostanes are discussed as a gold standard for the assessment of oxidative damages.

**Key words:** Lipid peroxidation, Malondialdehyde, 4-Hydroxy-2-nonenal, Antioxidant, Isoprostane

### INTRODUCTION

Lipid peroxidation is a free radical oxidation of polyunsaturated fatty acids (PUFAs) such as linoleic acid or arachidonic acid. The basic mechanism of lipid peroxidation is found in the radical chain reaction observed in typical autoxidation process, an oxidation by molecular oxygen ( $O_2$ ): initiation-propagation-termination (Porter, 1986). Therefore, lipid peroxidation is self-propagating and will proceed until substrate is consumed or termination occurs. In this way, lipid peroxidation is fundamentally different from other forms of free radical injury in that it is a self-sustaining process capable of extensive tissue damage (Porter *et al.*, 1995). In initiation step, abstraction of hydrogen at bisallylic position afforded lipid pentadienyl radical, from which various regioisomeric and stereoisomeric peroxy radicals are formed leading to a complex mixture of peroxy products (Scheme 1) (Marnett, 1999). The primary products of lipid peroxidation are lipid hydroperoxides (LOOH). They provide sources of a variety of reactive oxygen species (ROS) that cause oxidative stress along with other ROS such as superoxide ( $O_2^-$ ), ozone ( $O_3$ ), and hydroxyl radical ( $HO\cdot$ ) etc. There are also examples of enzyme-catalyzed oxidation of lipids where lipoxygenases (LOXs) (Brash, 1999) and cyclooxygenases (COXs) (Rouzer and Marnett, 2003;

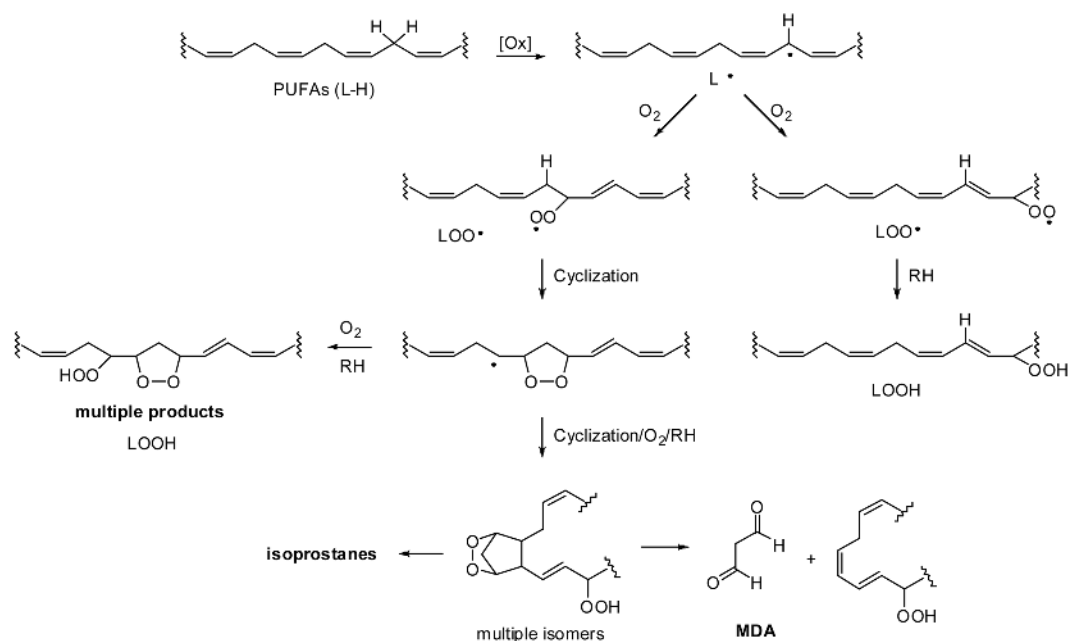
Rouzer and Marnett, 2009) are involved. When arachidonic acid (AA) is a substrate, isomers of HpETEs (hydroperoxy eicosatetraenoic acids) and HETEs (hydroxy eicosatetraenoic acids) are formed as primary lipid peroxidation products from LOX activity. COXs catalyze the formation of a rather specific hydroperoxide (prostaglandin (PG)  $G_2$ ) and hydroxy product ( $PGH_2$ ) from AA. In this review, non-enzymatic lipid peroxidation and the related toxicology will be discussed.

Lipid peroxidation has been implicated in various diseases and pathological conditions (Negre-Salvayre *et al.*, 2010) such as carcinogenesis (Marnett, 2000), cardiovascular diseases (Steinberg *et al.*, 1989; Glass and Witztum, 2001; Lee and Blair, 2001), neurodegeneration, (Bradley *et al.*, 2010; Markesbery *et al.*, 2005) and aging (Muller *et al.*, 2007).

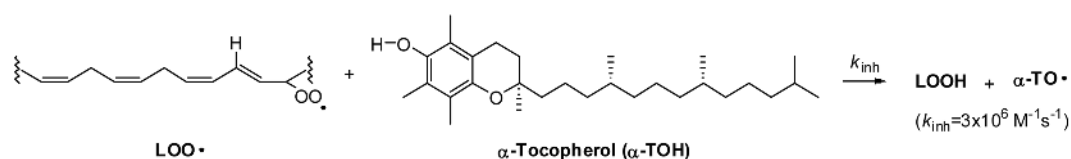
Phenolic antioxidants are typically used to inhibit lipid peroxidation. This class of antioxidants have a weak O-H bond dissociation enthalpy (BDE) such that it can transfer its hydrogen atom to lipid peroxy radical to perturb a radical chain reaction.  $\alpha$ -tocopherol ( $\alpha$ -TOH) is the most active phenolic chain-breaking antioxidants found in nature. Its O-H BDE (78.3 kcal/mol) is low enough to inhibit lipid peroxy radical ( $LOO\cdot$ ), a chain propagating species, from propagating the chain reaction (Scheme 2).

Many toxicological aspects of lipid peroxidation are attributed to various reactive aldehydes generated from oxidized lipids. These aldehydes react with cellular nucleophiles such as proteins, nucleic acids and glutathione (GSH)

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**Scheme 1.** Pathways of lipid peroxidation.



**Scheme 2.**  $\alpha$ -Tocopherol inhibits lipid peroxyl radical propagation.

to form a variety of adducts. The formation of those adducts are related to various pathologies. The reactive aldehydes make up a large part of endogenous electrophile repertoires that include malondialdehyde (MDA), 4-hydroxy-2-alknals (i.e., 4-hydroxy-2-nonenal (4-HNE)) and  $\gamma$ -ketoaldehydes (i.e., levuglandins). With tremendous advances in mass spectroscopy techniques coupled with reverse phase HPLC, detections and measurements of DNA adduct with lipid peroxidation products have been significantly progressed (Blair, 2008).

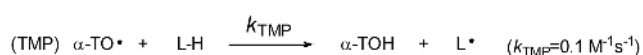
**DNA adduct formation and carcinogenesis.** Oxidative stress is well known to play an important role in carcinogenesis (Klaunig and Kamendulis, 2004; Jacobs and Marrett, 2010). Endogenous DNA adducts with reactive aldehydes generated from lipid peroxidation provide an important etiology of human genetic diseases and cancer. The DNA adducts lead to the alterations in DNA structure such as DNA base modifications and frame shifts. Pyrimido[1,2 $\alpha$ ]purin-10(3H)-one (M<sub>1</sub>G), a major DNA (dG)-MDA adduct, is one of the most important adducts and is found to be a strong mutagenic agent (Chaudhary *et al.*, 1996; Niedernhofer *et al.*, 2003). M<sub>1</sub>G induced frame shift mutations (-1 and -2) when posi-

tioned in a reiterated (CpG)<sub>4</sub> sequence but not when positioned in a nonreiterated sequence in *Escherichia coli* and in COS-7 cells (VanderVeen *et al.*, 2003). The spectrum of mutations induced by the naturally occurring M<sub>1</sub>G was determined by site-specific approaches using M13 vectors replicated in *Escherichia coli* (Fink *et al.*, 1997). In *E. coli*, M<sub>1</sub>G-modified genomes containing a cytosine opposite to M<sub>1</sub>G resulted in roughly equal numbers of M<sub>1</sub>G→A and M<sub>1</sub>G→T mutations with few M<sub>1</sub>G→C mutations. The total mutation frequency was approximately 1%, which represents a 500-fold increase in mutations compared with unmodified M13MB102 vector. Transformation of modified genomes containing a thymine opposite M<sub>1</sub>G allowed an estimate to be made of the ability of M<sub>1</sub>G to block replication (Fink *et al.*, 1997). Interestingly, this adduct can be even found in normal healthy individuals. It was found that DNA from healthy individuals contain about 5,400 MDA-dG adducts per cell in liver (Chaudhary *et al.*, 1994). This study demonstrated the normal endogenous level of DNA adduct formation caused by lipid peroxidation in disease-free subjects, and the crucial role of chemical carcinogenesis of endogenous source, antioxidants and DNA repair system. DNA adduct with 4-HNE (Minko *et al.*, 2009) was also found to

generate p53 gene mutation (Chung *et al.*, 2003) which can play a role in carcinogenesis.

Not only these DNA damages, i.e., mismatch or frame shift in DNA replication, caused by adduct formation, but also intracellular signal transduction can be caused by those reactive aldehydes. In particular, it is reported that 4-HNE inhibits the activation of NF- $\kappa$ B in human colorectal cancer cell line (RKO) and human lung cancer cell line (H1299). At doses that inhibit I $\kappa$ B $\alpha$  degradation, HNE inhibits I $\kappa$ B kinase (IKK) activity by direct reaction with IKK (Ji *et al.*, 2001). It was also found that activation of the heat shock response by HNE is dependent on the expression and nuclear translocation of heat shock factor 1 (HSF1), which promotes the expression of heat shock protein 40 (Hsp40) and Hsp70-1. HNE disrupts the inhibitory interaction between Hsp70-1 and HSF1, leading to the activation heat shock gene expression (Jacobs and Marnett, 2007). With increasing amount of data accumulated, there are attempts to deconvolute the biological activity and metabolic fates of these reactive aldehydes in systemic view. For example, oxidative damages on proteins by HNE (Codreanu *et al.*, 2009) and global gene expression change by HNE (West and Marnett, 2005) were investigated.

**Lipid peroxidation in LDL and chain-breaking antioxidants.** One of the most biologically important lipid peroxidations occurs in low-density lipoprotein (LDL). LDL is the major carrier of cholesterol and cholesteryl esters of fatty acids. Thus, the oxidation of LDL is accompanied by the extensive oxidation of linoleic acids and arachidonic acids (720 and 180 molecules per LDL, respectively) in LDL particle. Inference from epidemiological and biochemical studies suggested that the oxidation of LDL can lead to the generation of fatty streak, which has been believed an initial event of atherosclerosis. This oxidation theory suggested retentive and oxidative events trigger biochemical changes in artery wall that lead to lipid deposition. Since antioxidants such as vitamin E ( $\alpha$ -tocopherol) defend against oxidation of lipids (lipid peroxidation), vitamin E has been the focus of several large supplementation studies against atherosclerosis, but there is no clear link found between the vitamin and disease progression (Upston *et al.*, 2003). It was later found that  $\alpha$ -TOH is not a particularly good antioxidant in LDL. Indeed, under some conditions,  $\alpha$ -TOH in

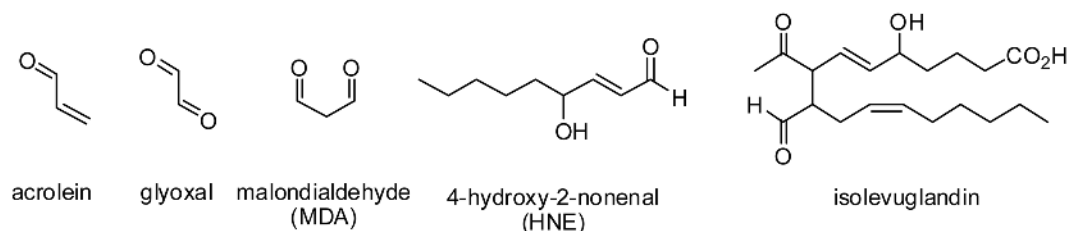


**Scheme 3.** Mechanism of  $\alpha$ -Tocopherol-mediated peroxidation (TMP).

LDL acts as a prooxidant to increase the oxidation of cholesteryl linoleate and phospholipids.  $\alpha$ -TOH can mediate lipid peroxidation through tocopherol-mediated peroxidation (TMP) mechanism (Bowry *et al.*, 1992) where tocopheroxyl radical ( $\alpha$ -TO $\cdot$ ) abstracts hydrogen atom from lipid molecule (L-H) to regenerate L $\cdot$ , the chain carrying radical, albeit slowly (Scheme 3). In fact, additional supplementation of  $\alpha$ -TOH from 18  $\mu$ M to 83  $\mu$ M resulted in about two-fold increase in the oxidation of both cholesteryl linoleate and phospholipids in the absence of co-antioxidants (Bowry *et al.*, 1992). Thus, can not provide protection against lipid peroxidation in atherosclerosis condition where extensive oxidative stress possibly deplete endogenous co-antioxidants that coordinate with lipophilic  $\alpha$ -TOH for the effective protection in LDL particles.

This finding shed a new light on the strategy for the development of novel chain-breaking antioxidant for the treatment of atherosclerosis. It is required that new antioxidant not only should have excellent antioxidant activity but also should not mediate TMP process. Indeed there are a few examples of remarkably successful antioxidants where pyridinol-based antioxidants inhibit the oxidation of cholesteryl linoleate in isolated human LDL, and do not participate antioxidant-mediated peroxidation (AMP) (Nam *et al.*, 2007; Kim *et al.*, 2005; Serwa *et al.*, 2010). Additional supplementation of lipophilic pyridinol antioxidants from 75  $\mu$ M to 150  $\mu$ M afforded about two-fold decrease in the oxidation of cholesteryl linoleate in the absence of co-antioxidants.

**Neurodegeneration.** Due to the high concentration of polyunsaturated fatty acids in brain relative to other organs, lipid peroxidation is one of the major outcomes of free radical-mediated injury to brain. Biochemical studies have demonstrated increased concentrations of reactive products from lipid peroxidation in diseased regions of Alzheimer's disease (AD) brain. For example, an elevated level of 4-HNE was found in ventricular fluid in AD (Lovell *et al.*, 1997) and acrolein (Lovell *et al.*, 2001) was also found at



**Fig. 1.** Reactive aldehydes generated from lipid peroxidation.

high level in AD brain, with toxic effect toward hippocampus. HNE was also found to bind to histones that provide a protective shield for DNA against oxidative stress, probably because of their abundant lysine residues, and this binding affects the conformation of the histone. This histone-HNE adduct affects the ability of the histone to bind to DNA leading to the vulnerability of DNA toward oxidation in AD brain (Drake *et al.*, 2004). Immunohistochemical studies have localized protein adducts of these aldehydes to lewy bodies in Parkinson Disease (PD) and dementia with lewy bodies (DLB), and to neurofibrillary tangles and some components of senile plaques in AD, DLB, and mouse models of A $\beta$  cerebral amyloidogenesis (Montine *et al.*, 2004). The enzymatic product of the COXs, PGH<sub>2</sub>, rearranges in part to highly reactive  $\gamma$ -ketoaldehydes, levuglandin (LG) E(2) and LGD(2). These  $\gamma$ -ketoaldehydes react with free amines on proteins to form a covalent adduct. It was found that LGE enhances oligomerization of amyloid  $\beta$  (1-42) thus increases neurotoxicity on primary cultures of cerebral neuron of mice (Boutaud *et al.*, 2006). The formation of LG adducts of protein (levuglandinyl-lysine adducts) in brains of AD patients showed that this post-translational modification is increased significantly in the hippocampus in Alzheimer's disease. The magnitude of the increase was correlated with the pathological evidence of severity (Zagolkapitte *et al.*, 2005).

There is growing evidence that oxidative stress (Adams and Odunze, 1991) and mitochondrial respiratory failure with attendant decrease in energy output are implicated in nigral neuronal death in Parkinson disease (PD) (Fessel and Jackson Roberts, 2005). Using polyclonal antibody to detect HNE-protein adduct in post-mortem brain, average of 58% of nigral neurons were positively stained for HNE-modified proteins in PD. In contrast, only 9% of substantia nigral neurons were positive in the control subjects. These results indicate the presence of oxidative stress within nigral neurons in PD, and this oxidative stress may contribute to nigral cell death. HNE-protein adducts are accumulated in age-related manner in the neuron (Yoritaka *et al.*, 1996).

A number of methods exist to quantify free radicals and their oxidation products although many of these techniques suffer from a lack of sensitivity and specificity, especially when used to assess oxidant stress status *in vivo*. Similarly, many oxidation products are not very good *in vivo* biomarkers for the assessment of oxidative damage mainly because of their chemical instability *in vivo* environment. More stable and quantitative biomarkers are isoprostanes (IsoPs), (Morrow *et al.*, 1990) isofuranes (IsoFs) (Fessel *et al.*, 2002) and neuroprostanes (NeuroPs) (Roberts *et al.*, 1998).

**Isoprostanes as a gold standard to assess oxidative stress.** Among the three biomarkers mentioned above, IsoPs are known to give the most reliable and robust results (Milne *et al.*, 2005, 2008). IsoPs are prostaglandin-like

compounds formed *in vivo* by non-enzymatic free radical oxidation of AA. In a recent multi-investigator study, termed the Biomarkers of Oxidative Stress (BOSS) Study, sponsored by the National Institutes of Health (USA), it was found that the most accurate method to assess *in vivo* oxidant stress status is the quantification of plasma or urinary IsoPs, and thus, currently, quantification of these compounds provides the "gold standard" to assess oxidative injury *in vivo* (Kadiiska *et al.*, 2005). Defining normal levels of F<sub>2</sub>-IsoPs in healthy humans (Milne *et al.*, 2007) is particularly important in that it allows for an assessment of the effects of diseases on endogenous oxidant tone and allows for the determination of the extent to which various therapeutic interventions affect levels of oxidant stress. Elevations of IsoPs in human body fluids and tissues have been found in a various human disorders, including atherosclerosis (Morrow, 2005; Pratico *et al.*, 1998; Davies and Roberts, 2011), diabetes (Davi *et al.*, 2003), obesity (Keaney *et al.*, 2003), cigarette smoking (Morrow *et al.*, 1995), neurodegenerative diseases (Montine *et al.*, 2004), and many others. Usefulness of IsoPs as biomarker can be originated from their biological activity in various oxidative stress settings. IsoPs strongly reduce the inflammatory response in macrophage by inhibiting lipopolysaccharide-stimulated I $\kappa$ B $\alpha$  degradation and subsequent NF- $\kappa$ B nuclear translocation and transcriptional activity (Milne *et al.*, 2005). Further, treatments for some of these conditions, including antioxidant supplementation, anti-diabetic treatments, cessation of smoking, and even weight loss, have been shown to decrease production of F<sub>2</sub>-IsoPs (Davi *et al.*, 1999; Roberts *et al.*, 2007). As an example for the assessment of oxidative damage, it was shown that doses of  $\alpha$ -TOH of 1600 IU/day or greater are required to statistically affect plasma F<sub>2</sub>-IsoP levels. Interestingly, vitamin C supplementation does not alter IsoP levels in humans (Roberts *et al.*, 2007; Levine *et al.*, 2001).

## CONCLUSIONS

Mechanism of lipid peroxidation process and formation of the oxidation products from oxidized lipids are discussed as well as their toxicological roles in various pathologies. Among the oxidation products, malondialdehyde and 4-hydroxynanenal have been a central theme not only because they extensively form adducts with DNA and proteins to alter their normal functions but also because they are found to be correlated with disease status *in vivo*. Their roles in controlling signal transduction pathways and gene expression profiles are being studied in global networking view, highlighting the importance of oxidative damages. As shown in atherosclerosis case study, supplementation of antioxidants might not be enough to ameliorate pathologies caused by oxidative damages. In spite of increasing body of biochemical and toxicological mechanisms and evidences in clinical pharmacology, there are not many successful thera-

peutic strategies developed to reduce oxidative damages other than antioxidant supplementation. Further investigations on the chemical, biochemical and biological mechanisms underneath the oxidative damages warrant the novel strategy to treat or prevent the oxidative damages.

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