Chapter

Lipid Peroxidation

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Abstract

Lipid peroxidation (LPO) is initiated by the attack of free radicals (eg OH ·, O2- and H2O2) on cellular or organelle membranes phospholipids or polyunsaturated fatty acids (PUFA), and with the formation of various types of aldehydes, ketones, alkanes, carboxylic acids and polymerization products. It is an autoxidation process that results. These products are highly reactive with other cellular components and serve as biological markers of LPO. Malondialdehyde (MDA), a toxic aldehyde end product of LPO, causes structural changes that mediate its oxidation, such as fragmentation, modification, and aggregation, especially in DNA and protein. The excessive binding of these reactive aldehydes to cellular proteins alters membrane permeability and electrolyte balance. Degradation of proteins leads to progressive degradation of the biological system mediated by oxidative stress. The chain reaction (CR) of LPO is initiated by the attack of free radicals on the PUFA of the cell membrane to form a carbon centered radical (R*). The O2 · - radical attacks the other lipid molecule to form lipid hydroperoxide (ROOH), thereby spreading the CR and forming the lipid peroxyl radical (ROO). These lipid hydroperoxides severely inhibit membrane functionality by allowing ions such as increased hardness and calcium to leak through the membrane. Damage to the lipid membrane and macromolecule oxidation can result in activation of necrotic or apoptotic tissue death pathways if severe enough.

Keywords: lipid peroxidation, free radical, malondialdehyde

1. Introduction

Free radicals are formed during the reactions required for the maintenance of normal metabolism and energy formation in biological systems. Under normal conditions, the most significant source of free radicals in cells is the leakage of electrons into molecular oxygen from electron flow in the mitochondria and endoplasmic reticulum during oxidative respiration. The superoxide anion formed in this way is converted into hydrogen peroxide, a reactive oxygen type. Hydrogen peroxide forms the peroxyl radical, which is the most reactive radical type in the organism in the presence of transition metal ions. When free radicals cannot be removed from the environment, they cause damage at the cell, tissue, and organ level by disrupting the structure of biomolecules such as lipids, proteins, and nucleic acids due to their high reactivity. Lipid peroxidation begins when polyunsaturated fatty acids (PUFAs), which are in the structure of membrane lipids in cells, are affected by free radicals. If peroxyl radicals are not cleaned, a chain reaction starts affecting the intact PUFA. Lipid peroxidation is damaging as it is a self-sustaining chain reaction. When lipid peroxides (LOOH) are broken down, aldehydes are formed, many of which are biologically active. These compounds are either metabolized at the cellular level or

diffuse from their initial domains and spread damage to other parts of the cell. When peroxidation of fatty acids containing three or more double bonds. Malondialdehyde (MDA) arises following the peroxidation of fatty acids comprised of three or more double bonds and appears in blood and urine. Due to its ability to corelate well with the degree of which lipid peroxidation occurs despite not being a specific or quantitative indicator of fatty acid oxidation, the measurement of MDA in biological material is used as an indicator of lipid peroxide levels. Nonenzymatic lipid peroxidation is a very harmful chain reaction. It both damages the membrane structure directly and also damages other cell components indirectly with the reactive aldehydes it produces. Thus, it causes tissue damage and many subsequent diseases.

2. Free radicals

According to quantum chemistry, only two electrons can enter the structure of a bond together. Electron pairs exist in a very stable state. Electrons in the human body exist almost entirely in electron pairs. When a bond breaks, the two electrons are either separated but remain in the same atom or both remain in the atom separately. If they remain together, the atom formed becomes an ion, and when they leave, the atom formed becomes a free radical [1]. Atoms or compounds that contain the unpaired electron in their final orbital are defined as free radicals. In other words, they are atoms or molecules that have an open electron shell configuration and contain an odd number of electrons in their structure [2]. The term Reactive Oxygen Species (ROS) is more commonly used in place of the term free oxygen radical as it includes molecules that are radical and are not actually radical, but that cause the formation of oxygen radicals with their reactions [1].

Despite oxygen being crucial for life, in some cases it can also damage cells. This damage is caused by increased oxygen-induced ROS production. The amounts of ROS produced under normal physiological conditions do not exceed the capacity of the natural antioxidant defense systems in the body. ROSs are chemical derivatives with unpaired high energy electrons in their outer orbits. In order to stabilize, ROS interact with any molecule they can find in their vicinity and exchange electrons. Molecules that react with free radicals turn into free radicals and initiate the damage chain reaction. These radicals react with organic and inorganic chemicals such as protein, lipid, and carbohydrate. When radicals occur in cells, they react with nucleic acids and various membrane molecules and break them down. While radicals affect intracellular organelles, they also create distant effects by passing to the extracellular compartment [1]. Although oxygen is crucial for human life, some ROS that occur during normal metabolism have the potential to cause great harm to the body. Compared to normal oxygen molecules, ROS, which are mostly composed of free radicals, appear as oxygen forms with higher chemical reactivity [3].

Free radicals are any atom or molecule with one or more unpaired electrons produced in many physiological or pathological conditions. These molecules, also known as oxidant molecules or reactive oxygen particles, easily exchange electrons with other molecules [4]. A compound can return to a free radical by losing an electron (reduction) or gaining an additional electron (oxidation). Free radicals can be part of a larger structure, as well as in immobile or small and freely spreading species [5–8]. Free radicals are frequently produced by the mitochondria during the body's normal use of oxygen. These free radicals, which are formed as a result of energy production, can change the structure of lipids, proteins, and nucleic acids. Free radicals are produced from many endogenous and exogenous sources as well as mitochondria and cause a variety of damage alongside their benefits. The benefits of free radicals only occur when they are of low concentration. Low concentration

free radicals are involved in the activation of cellular signals such as calcium release from intracellular stores, and the activation of tyrosine phosphating and growth factor signals, along with defense functions such as defense against infections, the killing cancer cells and detoxification of xenobiotics [9].

3. Ways in which free radicals form in the cell

Common biochemical events, such as those which occur during normal respiration, cause reduction–oxidation (redox) reactions. The molecular oxygen in mitochondria is gradually diminished with the adding of four electrons to form water. Several toxic intermediate derivatives occur during this event. These include superoxide radicals (O^{-2}), hydrogen peroxide (H_2O_2) and hydroxyl (OH^-). In addition, some intracellular oxidases such as xanthine oxidase directly form superoxide radicals as a result of their activities. It catalyzes the formation of free radicals (Fe^{+++} $H_2O_2 \rightarrow Fe^{++++}$ OH^- + OH^-) as in the Fenton reaction by exchanging free electrons during some intracellular reactions in exchange metals such as copper and iron. To play a part in the Fenton reaction, the intracellular free iron, occuring in the ferric state (Fe^{+++}), must initially be reduced to its ferrous (Fe^{++}) form. Iron and superoxide are both required for maximum oxidative cell damage as the reduction is amplified by the superoxide ion.

By absorption of radiant energy (such as ultraviolet light, X-rays): For example, water can be hydrolyzed to hydroxyl (OH^-) and hydrogen (H^+) free radicals with ionizing radiation.

By the intracellular enzymatic metabolism of external chemicals or drugs: For example, CCl_3 free radical is formed as a result of the intracellular metabolism of carbon tetrachloride (CCl_4).

Nitric oxide (NO), an important chemical mediator normally synthesized in various cell types, reacts with oxygen, especially non-radical peroxynitrite, a type of free oxygen that inhibits mitochondrial respiration, as well as the radical nitrogen dioxide (NO2) and nitrogen trioxide (NO3) [1]. Common free radicals, their symbols and identities are shown in the table below (**Table 1**):

Free radical	Symbol	Identity
Hydrogen	H	The simplest radical.
Superoxide	$O_2^{\cdot -}$	The first intermediate product of oxygen metabolism.
Hydroxyl	$OH^{\cdot}$	The most toxic (reactive) oxygen metabolite radical.
Hydrogen peroxide	H_2O_2	Reactivity is very low, molecular damage ability is poor.
Singlet oxygen	O_2^-	Strong oxidative form of oxygen with fast half-life.
Perhydroxy radical	$HO_2^{\cdot}$	Rapidly dissolves in lipids and increases lipid peroxidation.
Peroxide radical	ROO^-	is less effective than perhydroxyl, localized to lipids.
Trichloromethyl	CCl_3	CCl_4 is a radical produced in the liver, the product of metabolism.
Thiyl radical	$RS^{\cdot}$	General name for sulfurous and unpaired electron-containing species.
Alkoxyl	$RO^{\cdot}$	Oxygen metabolite produced by the breakdown of organic peroxides.
Nitrogen oxide	NO	is produced in vivo from the amino acid NO L- arginine.
Nitrogen dioxide	NO_2	is produced by the reaction of NO with oxygen [10].

Table 1.
Free radicals, symbols, and identities.

The most important free radicals that occur are:

- Superoxide radical (O_2^-).
- Hydrogen peroxide (H_2O_2).
- Hydroxyl radical (HO^-).
- Singlet oxygen ($O_2\uparrow\downarrow$) [11, 12].

3.1 Superoxide radicals (O_2^-)

The superoxide anion radical (O_2^-) is produced by the single electron reduction of oxygen which acts as an intermediate in a number of biochemical reactions in body [13] and is a weak oxidant that cannot cause serious cell damage by itself.

However, it may lead to the initiation of a series of reactions that can lead to oxidative stress [6, 14, 15]. One of the main points of superoxide production is Coenzyme Q, and this anion is formed at other points in the electron transport chain as well as in the mitochondrial electron transport chain. Another ROS is produced by the O_2^- radical, which does not leak far from where it originates [12, 16].

$$\begin{aligned} &O_2 + \acute{e} \rightarrow O_2^- \left(\text{superoxide radical}\right) \\ &H_2O_2 + O_2 \rightarrow HO^- + OH^- + O_2 \end{aligned} \tag{1}$$

The OH^- radicals produced are highly reactive and can cause significant damage by reacting with structures such as DNA [6, 17, 18].

The half-lives of superoxide radicals that produce H_2O_2 and oxygen by the dismutation reaction are quite short. This reaction occurs spontaneously and is catalyzed by the Superoxide Dismutase (SOD) enzyme [6].

$$O_2^- + O_2^- + 2H + SOD \rightarrow H_2O_2 + O_2 \tag{2}$$

In natural conditions, O_2^- can be produced in muscle tissues in a variety of ways. One of the sources of O_2 in muscle tissues are various components of the electron transport chain in mitochondria, such as NADPH-linked dehydrogenase and ubiquinone, which can leak electrons into O_2. Autoxidation of heme proteins [19, 20] and metabolic enzymes such as xanthine oxidase [21] are other sources of O_2. With the ingestion of bacteria, the activation of several leukocytes in the vasculature of the muscle tissue causes the production of O_2^-, one of the major bactericides [22].

3.2 Hydrogen peroxide (H_2O_2)

Aerobic cells naturally contain low concentrations of hydrogen peroxide (H_2O_2) as a metabolite. In an O_2 forming system, it is expected to give H_2O_2 catalyzed by nonenzymatic or superoxide dismutase (SOD) [23]. Although it is not free radical, hydrogen peroxide reacts with a transition metal (e.g. Fe^{+2}) to form a free radical [16].

H_2O_2 production has been detected in mitochondria, microsomes, peroxisomes and phagocytic cells. Also, many enzymes, such as xanthine oxidase, aldehyde oxidase, urate oxidase, glucose oxidase, glycolate oxidase, and D-amino acid oxidase,

can directly produce H_2O_2 [23]. It has been reported that H_2O_2 is produced at a hemoglobin rate of approximately 3.9 × 10-9 M/hg and the concentration of H_2O_2 in red blood cells in a steady state is 2 × 10-10 M [24]. It has been reported that H_2O_2 formed during oxidation of oxymyoglobin plays an important role in lipid peroxidation [25]. Furthermore, it was reported that turkey muscle tissues stored at 37°C for 30 minutes produced approximately 14.0 nmol H_2O_2 per gram fresh weight, and its formation increased with storage at 4°C [26].

H_2O_2, which lacks unpaired electrons, is not a radical and, unlike charged O2, shows limited reactivity and permeability to the membrane [27]. Nevertheless, H_2O_2 can have devastating effects by generating more reactive species such as OH by catalysis of Fe (II) [28]. In addition, H_2O_2, depending on its concentration, can denature heme proteins to release iron and heme group or to convert heme protein to ferryl or perferryl radical [20].

3.3 Hydroxyl radicals (HO^-)

The hydroxyl radical (OH) is the most reactive oxygen radical [29]. It is the most powerful free radical hydroxyl radical found in biological systems. In tissues exposed to radiation, a large part of the energy is absorbed by the water inside the cell and the radiation creates a covalent bond between oxygen and hydrogen, forming hydrogen (H^-) and hydroxyl radical (OH^-).

$$H - O - H \rightarrow H^- + OH^- \left(\text{Hydroxyl radical}\right) \tag{3}$$

OH^- radicals, which can provide radical formation and participate in a series of reactions, cause strand breaks in DNA by joining the structure of bases in DNA and RNA, which they do by causing a lot of damage to the bases and sugars of DNA. If the damage is very severe, it may not be repaired by cellular protective systems and as a result, mutations and cell death occur [14, 17, 30].

The steady-state concentration of the OH^- radical in vivo is zero because it reacts with every molecule in the living cell, such as DNA, protein, phospholipid, amino acid, and sugar, at or near the place of formation. The high reactivity of the OH^- radical is thought to result from the extraordinary combination of three properties. These properties include high electrophilicity, high thermochemical reactivity, and the ability to form near target molecules [31]. OH^- formation was achieved in living erythrocytes under the effect of adriamycin using the spin trap electron paramagnetic resonance (EPR) technique [32]. Most of the OH^- produced in vivo or in situ was obtained from the decomposition of H_2O_2 [33] by Fe (II) catalysis. Additionally, OH^- can be produced by various sources: sunlight (Joseph JM, Aravindakumar), ultraviolet radiation [34], ionizing irradiation [35], reaction of hypochlorous acid with O2- [36] and sonolysis of water (ultrasound) [37].

The reaction of the OH^- radical can be inhibited by OH^- scavengers such as methanol, ethanol, 1-butanol, mannitol, formate, thiourea, dimethylthiourea, glucose, tris-buffer, or sorbitol [23]. Although OH^- scavengers prevent OH^- from reacting with other molecules, including lipid molecules, they are not always effective. There are several reasons to consider:

1. Reaction of the OH^- radical with a scavenger can create scavenger radicals that can react with other molecules in the system [38].

2. More attention has been paid to the possibility of a metal-mediated mechanism [28]: OH^- produced by the reaction of H_2O_2 with metal ions bound to

macromolecules can react with metal-binding molecules. It has been reported that as a result of the formation of the Fe (II) ion and 2-deoxyreebose complex, the Fe (II) ion that binds to DNA interacts with H_2O_2 to form OH^-, which instantly damages DNA [39]. It was determined that the Fe (III) ion binds to the membrane and then forms free radicals in the binding site. It has been suggested that iron is accepted as the main binding site of the sulfone group with the carboxyl groups of sialic acids to the membrane, the sulfate group of glycolipids and the phosphate head group of glycoproteins and phospholipids [40]. On the other hand, it has also been reported that OH^- scavengers effectively inhibit OH^- formation in the presence of EDTA. Indeed, EDTA allows Fe (II) ions to be removed from these binding sites [41]. Thus, the toxicity of O_2 and H_2O_2 may be due to the presence and distribution of metal ion catalysts to form OH^- in cells.

3.4 Singlet oxygen ($O_2\uparrow\downarrow$)

Singlet oxygen is the name given to the excited form of oxygen; it is a reactive oxygen type with a very high non-radical reactivity. By directly reacting with unsaturated fatty acids, it forms the peroxyl radical and initiates a lipid peroxidation as strong as the hydroxyl radical [10].

4. Free radical sources

Continuously produced SR in the cell and in the environment can be generated by both endogenous and exogenous sources.

Endogenous (Natural) Sources:

- Oxygen catalyzed by the electron transport system during oxygen respiration in mitochondria produces free radicals as a by-product.
- In case of inflammation, cytokines are released and as a result neutrophils and macrophages begin to produce free radicals.
- Free radicals can originate from a variety of sources such as lipid peroxidation, xanthine oxidase, and mitochondrial cytochrome oxidase.
- Free radicals can be produced by smooth muscle cells, platelets, and arachidonic acid metabolism.
- It may occur as a result of electron leaks in the Cytocom p450 system in the endoplasmic reticulum with enzymes such as xanthine oxidase (XO) and nicotinamide adenine dinucleotide phosphate (NADPH) oxidase during autooxidation reactions.
- Stress from mental stress or body fatigue can create free radicals as a toxic by-product. In addition, hormones such as cortisol and catecholamine can cause stress reactions in the body, and these hormones themselves can turn into free radicals.
- Immune system cells can generate ROS and oxy-radicals in response to pathogens.

Exogenous Sources:

- X-rays, UV rays, microwave rays, gamma rays.
- Burning organic materials during cooking.
- Volcanic activities, forest fires.
- Pollutants such as benzene, asbestos, formaldehyde, carbon monoxide, toluene, and ozone.
- Chemicals such as glue, cleaning products, thinner, paint, pesticides, and perfumes.
- Water contaminants such as chloroform and other trihalomethanes.
- Cigarette smoke, exhaust smoke, alcohol and cigarette use can contribute to the production of free radical exogenously [9, 42–45].

5. Damage associated with free radicals

As a result of the damaging effects of free radicals on cells, the cell's plasma and organelle membranes lose their continuity. As a result, sodium and calcium ions enter the cell in addition to water. Morphologically recognized by their pale granular cytoplasm, these cells swell. Over time, this structural defect leads to irreversible changes in the cell0, followed eventually by death [1].

5.1 Lipids

Lipids are the most sensitive biomolecules to the effects of free radicals, and the unsaturated bonds of fatty acids and cholesterol in cell membranes react very easily with free radicals to form peroxidation products. The oxidative breakdown of polyunsaturated fatty acids, known as lipid peroxidation and which is highly harmful, proceeds as a self-sustaining chain reaction [17, 46]. Lipid peroxides, which are an important component of cell membranes, form RS- and ROO- radicals with the presence of transition metals such as Fe and Cu. In this way, Fe and Cu salts increase the rate of lipid peroxidation and consequently reduce the fluidity and permeability of the cell membrane and cause the disruption of membrane integrity [6, 17, 47].

5.2 Lipid peroxidation

Lipid peroxidation is a free radical chain reaction that is comprised of three primary steps: initiation, propagation, and termination. Highly reactive radicals, such as the hydroxyl radical, attack polyunsaturated fatty acids, causing a hydrogen atom to be removed from the methylene (—CH_2—) group and, thus, initiate lipid peroxidation. Polyunsaturated fatty acids are very sensitive to peroxidation, as the number of double bonds in the fatty acid side chain increases, the hydrogen atom cleavage becomes easier [13]. Conjugated dienes will, however, react to one another in the bounds of the membranes or other membrane components such as protein and cholesterol under conditions when O_2 is extremely restricted [48]. The creation of conjugated dienes is followed by changes in the structure of the double bond from cis to trans form,

which may facilitate tighter packing of the unsaturated fatty acids, contributing to the development of more rigid domains inside the bilayer of oxidized lipid [49].

When the hydrogen atom leaves the molecule by acquiring an electron, only one electron remains in the carbon of the fatty acid; In order to eliminate the weakening of the C-H bond in the carbon atom adjacent to the double bond, the carbon-centered radical forms the conjugated diene. Conjugated diene reacts with oxygen, causing the lipid peroxyl radical (LOO•); the lipid radical formed in this step is important because it starts a chain reaction by removing the hydrogen atom from another fatty acid. Peroxyl radicals show less reactive properties than •OH; however, they can reach farther regions. Peroxyl radicals can react with each other, attack membrane proteins or break hydrogen atoms from neighboring fatty acid chains, leading to the progression of lipid peroxidation chain reaction. Lipid peroxidation in biological membranes can lead to decreased membrane fluidity and membrane potential, increased permeability to H^+ and other ions, and disruption of organelle or cell integrity [13].

The termination process is the last stage of lipid peroxidation. During this process, LOOs either undergo a reciprocal causal nexus or self-destruct and in this way go on to form non-radical products. Despite their potential to breakdown when exposed to high temperatures or by contact with transitional metal ions, LOOH is a compound which remains stable at physiological temperatures [23]. The formed free radicals (LO•, LOO•) and electrophilic products (e.g. 4-hydroxynonenal) can react with neighboring membrane proteins as well as diffuse with distant molecules such as DNA [13].

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