REVIEW ARTICLE

Lipophilic fluorescent products as a potential biomarker of oxidative stress: A link between central (brain) and peripheral (blood)

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Oxidative stress plays a key role in brain damage because of the sensitivity of brain tissue to oxidative damage. Biomarkers with easy measurement can be a candidate for reflecting the oxidative stress issue in humans. For this reason, we need to focus on specific metabolic products of the brain. End products of free radical reactions such as malondialdehydes form fluorescent products known as lipophilic fluorescent products (LFPs). The distinctive feature of LFPs is their autofluorescent properties. LFPs are detectable in the brain and cerebrospinal fluid. Furthermore, because of the diffusion into the bloodstream, these lipophilic molecules can be detected in the blood. Accumulations of these compounds produce more reactive oxygen species and increase the sensitivity of cells to oxidative damage. Hence, LFPs can be considered a danger signal for neurons and can be introduced as a strong index of oxidative damage both in the central and in the peripheral.

Key words: Brain, fluorescence, lipid peroxidation, malondialdehyde, oxidative stress

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INTRODUCTION

Today, the incidence and prevalence of brain diseases such as neurodegenerative disorders are increasing. The involvement of oxidative damage caused by free radicals (FRs) in the pathogenesis of brain disease is well known.[1-3] Reactive oxygen species (ROS) are produced in normal cell physiology in connection with normal cellular processes such as cellular metabolism and mitochondrial respiration. There is a balance between the production of FRs and their elimination under normal conditions, so that if the production of these species is more or there is a disturbance in the cellular defense mechanism, these active species can damage vital macromolecules including lipids, proteins, and nucleic acids and may lead to cell death.[4] In addition, ROS react with various reactive compounds in biological systems, leading to molecular injury.[5,6]

On the other hand, it has been proven that the brain is highly sensitive to oxidative stress due to specific biochemical, physiological, and anatomical reasons such as (i) high oxygen consumption, (ii) rich in unsaturated fatty acids, and (iii) low levels of antioxidants.[4] Therefore, it is necessary to maintain the homeostasis of oxidation–reduction state for brain cells.[7] A change in the state of oxidation–reduction of the cell due to the increase of oxidants or the lack of antioxidant system leads to the production of ROS, and the accumulation of these species also causes damage to the mitochondria and finally to the start of neuron destruction[8] [Figure 1]. Specifically, elevations in lipid peroxidation markers in both brain and body fluids have been observed in several brain diseases, comprising Alzheimer's disease, Parkinson's disease, multiple sclerosis, amyotrophic lateral sclerosis, and Huntington's disease.[9-11] In support of these findings, several other reports have reported increased levels of lipid peroxidation reactive products in brain regions with other disease conditions.[9,12,13]

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Mediators of lipid peroxidation, including malondialdehyde (MDA), can damage polyunsaturated fatty acids (PUFAs) in cell membranes and produce lipophilic fluorescent products (LFPs). The distinctive feature of these compounds is their autofluorescent property, which has made these compounds identifiable by means of the fluorescent evaluation technique at different wavelengths. These fluorescent compounds, as indigestible substances, participate in the damage and loss of function of biological macromolecules, especially in neurons, and cause disruption in neuron function and homeostasis. Therefore, these compounds can be considered a danger signal for neurons.[14] It has been suggested that autofluorescent biological products can be used as fingerprints to track oxidative damage processes and may pave the way for the diagnosis of disease states. Therefore, the examination of fluorescent oxidation products can be a reliable and appropriate approach to evaluate oxidative stress in tissues and blood in laboratory and clinical studies.[14]

The main goal of this review is to introduce an indicator of cellular oxidation state that has the following characteristics: i) it can be easily evaluated by fluorescent technique; ii) it is stable; iii) it can be applied in human studies on a large scale; iv) predict the damage caused by oxidative processes both in the central (brain tissues and CSF) and in the peripheral (blood and other tissues).

LIPID PEROXIDATION TRIGGERS THE FORMATION OF LIPOPHILIC FLUORESCENT PRODUCTS OF FREE RADICAL REACTIONS

Lipid peroxidation includes a series of reactions in which the production of FR occurs selectively in the lipid components of the cell membrane. For example, PUFAs are highly sensitive to damage made by ROS which is found in complex lipids, e.g., phospholipids.[13] PUFAs are easily subjected to lipid peroxidation chain reactions and produce highly reactive aldehydes, including acrolein, MDA, 4-hydroxy-2-nonenal (4-HNE).[14,15] The two markers that are used most extensively to evaluate lipid peroxidation are MDA and 4-HNE.[15-17] Chio and Tappel[18] showed for the first time that MDA, as a lipid peroxidation product, reacts with free amino groups to produce fluorescent pigments. Interaction of the carbonyl groups of unsaturated aldehydes with amino groups of various biomolecules including proteins, amino acids, DNA, and phospholipids results in forming of Schiff bases.[19] The final products of these reactions were early called lipofuscin-like pigments due to their similarity to the fluorescent characteristics of the aging pigment lipofuscin.[20] In addition, 4-HNE is also a reactive aldehyde formed throughout lipid peroxidation, undertaking fluorescent compound formation during reaction with amino groups.[21] Many studies have shown

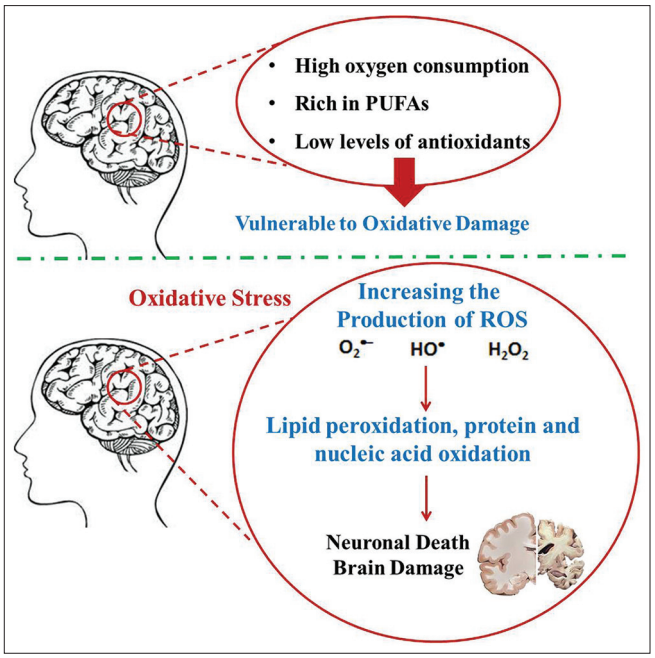

Figure 1: Oxidative stress caused increased production of free radicals in the brain. Increased reactive oxygen species results in the initiation of lipid peroxidation, protein, and nucleic acid oxidation and finally leads to neuronal death and the onset of brain damage. ROS = Reactive oxygen species

that aldehydes contributing to LFP generations are 4-HNE and MDA.[22,23] In our recent findings, enhancement of lipid peroxidation markers, for instance, MDA as an oxidative stress biomarker, was observed, demonstrating that peroxidation of lipids occurs during the advancement of LFP production.[14,24,25]

CLASSIFICATION OF LIPOPHILIC FLUORESCENT PRODUCTS

There are three types of LFPs based on solubility, including lipid soluble, water soluble, and insoluble.

First, the lipid-soluble LFP compounds found in lipid extracts of tissues are mainly derived from phosphatidylethanolamine and phosphatidylserine, which react with carbonyls taken from unsaturated lipids.[26] Fletcher *et al*. have proposed a susceptible fluorometric experiment for lipid-soluble LFP measurement accumulated in the tissues. In this completely easy and reliable fluorometric method, the desired compounds are extracted from tissues by chloroform–methanol (2:1). In the tissues, most of the pigments come into the organic solvent stage.[27] It has been documented that lipid-soluble LFPs can be evaluated with maximum excitation within the range of 340–370 nm and maximum emission within the range of 420–470 nm.[26] Due to the difference of PUFAs in different tissues, the spectral pattern of these compounds is tissue-specific and different. Therefore, the lipid extracts' fluorescent evaluation can be used as a parameter to

measure the state of cellular oxidation–reduction in tissues *in vivo*.

The second class of LFPs belongs to water-soluble pigments, which refer to pigments that are extracted from homogenized tissue in the aqueous methanol phase and extracted from tissue homogenates with chloroform–methanol–water. It has been demonstrated that aqueous extracts of tissues are not suitable for fluorescent evaluation, because in this method, interfering fluorescent compounds including flavins, NADH, and NADPH are hard to eliminate. Hence, the aqueous extracts are suitable candidates for an *in vitro* environment.[26]

Finally, according to the biochemical observations, insoluble LFPs have suggested that large amounts of LFP pigments remain in the insoluble part of the tissue extracts. The insoluble LFPs likely arose from primary amines insoluble in organic solvents such as proteins, nucleic acids, and some amino acids that react with carbonyls taken from lipid peroxidation. It has been suggested that the investigation of these pigments is restricted to an *in vitro* environment.[26]

CHEMICAL STRUCTURE OF LIPOPHILIC FLUORESCENT PRODUCTS

Active carbonyl aldehyde moieties through cross-linking with amino groups produce LFPs that represent a mixture of several different known and unknown compounds, as each has its chemical structure. Fluorescent pigments in the laboratory environment can best be described by incubating aldehydes taken from lipid peroxidation, including MDA, with free amino compounds.[28] Based on several experimental studies, it has been proven that MDA is involved in the formation of LFPs. Chio and Tappel have shown that MDA reacts with amino acids or their esters and n-hexylamine under acidic conditions to produce fluorescent chemical compounds that have spectra in which excitation maxima were in a range between 370–400 nm and 450–470 nm for emission maxima.[18,29] The basic analysis showed that these compounds are conjugated with Schiff bases with structure –N=CH–CH=CH–NH–.[30] Furthermore, the Schiff bases synthesized from amines and aldehydes with a focus on the structural requirement for fluorescence revealed that the fluorescent compounds produced throughout lipid peroxidation are obtained from MDA mainly because of the -NH- group conjugated with the imine as a powerful electron donor.[31] Targeting the amine group of macromolecules by MDA leads to generating LFPs with aminoiminopropene chemical structure (–N=CH–CH=CH–NH–)[32] and 1,4-dihydropyridine-3,5-dicarbaldehyde structure.[33,34]

NATIVE FLUORESCENT CHARACTERISTICS OF LIPOPHILIC FLUORESCENT PRODUCTS

Scientists' attention to autofluorescence has been accompanied by technological advances in spectrofluorometry since the 1900s, and over the years, research and application of autofluorescence in biological and biomedical diagnostics have been reviewed.[35] In recent years, autofluorescence spectroscopy or native fluorescence spectroscopy is one of the methods that have attracted attention for the study of cells in general biology.[36] It has been suggested that bioproducts of autofluorescence can be used as fingerprints to track pathological processes and can be used to diagnose disease states.[37]

The distinctive feature of LFPs is their autofluorescent property due to having aromatic groups or cyclic molecules with several covalent bonds, which has made these compounds identifiable using the fluorescent evaluation technique at different wavelengths in their specific excitation and emission ranges.[28] Since the autofluorescent property of LFPs is similar to the autofluorescent property of lipofuscin pigment, these compounds are also called "lipofuscin-like pigments."[38] Fluorescent chemicals produced in such reactions are generally a mix of individual compounds which have fluorescence features.[28] In other words, the composition of fluorescent products is determined by the kind of ROS that produces them and is particular to each tissue.[39]

ADVANTAGES OF LIPOPHILIC FLUORESCENT PRODUCTS AS AN OXIDATIVE STRESS BIOMARKER

Recent evidence suggests that scientists are seeking to identify stable biomarkers that can be easily measured and represent systemic oxidative stress and can also be used in large-scale human studies.[40,41] According to reports, LFPs are used as indicators of oxidation–reduction status.[14,24,25,42-44] Hence, this evaluation is ten to a hundred times more sensitive than commonly used oxidation measurements.[45,46] One of the advantages of this marker compared to the other oxidation ones including MDA is that the fluorescent assay potentially reflects the oxidation products of some other original containing lipids, proteins, and nucleic acids.[40,46-48] Moreover, they are the final products of oxidation, and hence, they are stable products and are also produced in humans over time during the aging process.[37,49]

ELIMINATION OF LIPOPHILIC FLUORESCENT PRODUCTS FROM CELLS

Activation of phagocytes has been shown to dissolve and remove LFPs.[50] Spoerri and Glees have shown that

in the brain, phagocytes participate in the deletion of LFPs.[51] Furthermore, the enhancement of antioxidant enzyme activity may exert an effect on LFPs to reduce their formation, because lipid peroxidation, which probably plays a remarkable role in the formation of LFPs, is stopped by the mentioned enzymes.[52]

ACCUMULATION OF LIPOPHILIC FLUORESCENT PRODUCTS IN CELLS

The mechanism associated with the accumulation of LFPs in the elderly is not obvious. However, it has been suggested that young animal cells have a special capability to reuse waste materials produced in them since this efficacy may be decreased in the elderly.[53] Due to the constant chemical structure of LFPs, their accumulation in cells becomes advanced and changeless with aging.[50] The autophagy-lysosome pathway (the main cell clearance machine) is involved in the degradation of waste materials and aged proteins. Disturbances in this pathway cause protein accumulation, the production of toxic protein species, and the accumulation of dysfunctional organelles.[54] Theoretically, if the autophagy process is stopped, the accumulation of LFPs occurs over time gradually. On the contrary, the stimulation of this process results in increased performance of degradation of newly formed LFPs and less accumulation of LFPs.[55] The findings of Lei *et al*.'s study showed that the inhibited autophagy caused by 3-methyladenine in cultured retinal cells caused the accumulation of autofluorescent products, while the stimulation of autophagy by rapamycin in these cells reduced autofluorescent products.[55] Also, Stroikin and his colleagues have reported that inhibition of autophagy in cultured fibroblast cells leads to the accumulation of autofluorescent compounds.[56] This funding shows that autophagy dysfunction can play a role in the accumulation of autofluorescent products in cells, but more extensive studies are needed.

On the other hand, it has been seen that the accumulation of these compounds causes disturbances in cell function and homeostasis, producing more ROS, and increased sensitivity of cells to oxidative damage. The indigestible character of this substance is associated with a gradual decrease in lysosomal function and changes in the processes of phagocytosis and autophagy, which have secondary effects on many different cellular activities. Furthermore, these compounds can cause the liberation of inflammatory cytokines and the activation of macrophages and lead to persistent inflammatory-oxidative processes. Therefore, these compounds can be considered a dangerous signal for cells.[57] Importantly, the degree of accumulation is linked with oxidative stress and damage, as well as with essential cellular dysfunction, which is known to contribute to many age-related disorders.[58] The progressive reduction, reduced adaptation, and an increased likelihood of disease and death for the organism inevitably resulted in these alternations at the cellular level.

LIPOPHILIC FLUORESCENT PRODUCTS AS A LINK BETWEEN CENTRAL (BRAIN) AND PERIPHERAL (BLOOD)

Redox status changes have been observed in the early stage of brain damage and are not limited to brain tissue.[59] Several studies have shown that the permeability of the blood–brain barrier is elevated in brain disorders.[60,61] While clinically used biomarkers exist in CSF, this method has several limitations.[62] Consequently, reactive compounds such as MDA and LFPs in the brain can diffuse to the blood and attack PUFAs in membranes of erythrocytes and proteins of plasma resulting in the formation of LFPs in the blood.[63] Therefore, the fluorescent products are not only produced in the brain tissue but also can be accessed and estimated in CSF and blood as well[14] [Figure 2]. These products represent the constant final products of ROS rush and can be used as indicators of oxidative damage to cells. Compared to spinal tap for CSF sampling, blood sampling is less invasive, not more expensive, and practical. Therefore, finding a reliable blood or plasma marker for oxidative stress would be extremely desirable.[14] Since oxidative stress is involved in many diseases, LFPs can be used as a useful marker of oxidative stress in epidemiological studies. However, so far, this method has not been extensively used in human studies.[64]

HOW CAN PATHOLOGY AFFECT THE GENERATION OF SEVERAL FLUORESCENT COMPOUNDS IN TISSUES?

Pathological conditions can stimulate the generation of several fluorescent compounds in tissues in two ways. The first way is that there is an increase in the amount of fluorescent compounds before the onset of pathology. For instance, there is an electron leak in the mitochondria of healthy individuals, which leads to the production of a certain basal level of fluorescent compounds. If the onset of pathology is accompanied by more electron leakage, such as when peroxidation of lipids increases, the composition of fluorescent compounds does not change, although the concentration of compounds increases. This type of process can explain the slight elevation in fluorescent compounds. A second possibility is that the formation of fluorescent compounds is caused by new types of FR attacks. For example, a cellular structure may be affected by FR attacks and lead to the generation of a new type of fluorescent compound with a different composition that is not present in healthy individuals.[64] However, in both cases,

fluorescent products have a high potential diagnostic value; thus, it is essential to recognize the chemical composition of these compounds by the high-performance liquid chromatography technique.

APPLICATION OF LIPOPHILIC FLUORESCENT PRODUCT ANALYSIS IN STUDIES IN RELATION TO BRAIN DISEASES AND OTHER DISEASES IN DIFFERENT TISSUES

Since LFPs are introduced as indicators of oxidative damage in lipids and other molecules caused by ROS, their measurement may be a useful implement to monitor pathological processes related to oxidative stress. Disruption of biological molecules occurs following excessive production of ROS, which is involved in the pathophysiological mechanism of many disorders.[28] A large number of studies show that during oxidative stress, the structure of biological molecules is damaged and converted into LFPs. Most of the available information about LFP assessments as an indicator of oxidative stress in various studies in relation to brain diseases and other diseases are listed in Tables 1 and 2, respectively.

CONCLUSION

Since oxidative stress has been hypothesized to be critical in brain damage, LFPs can be a useful marker of oxidative stress. The fluorescent assay strongly measures oxidation products from multiple sources including lipid, protein, and DNA and thus may reflect more global oxidative stress than a single oxidation product.

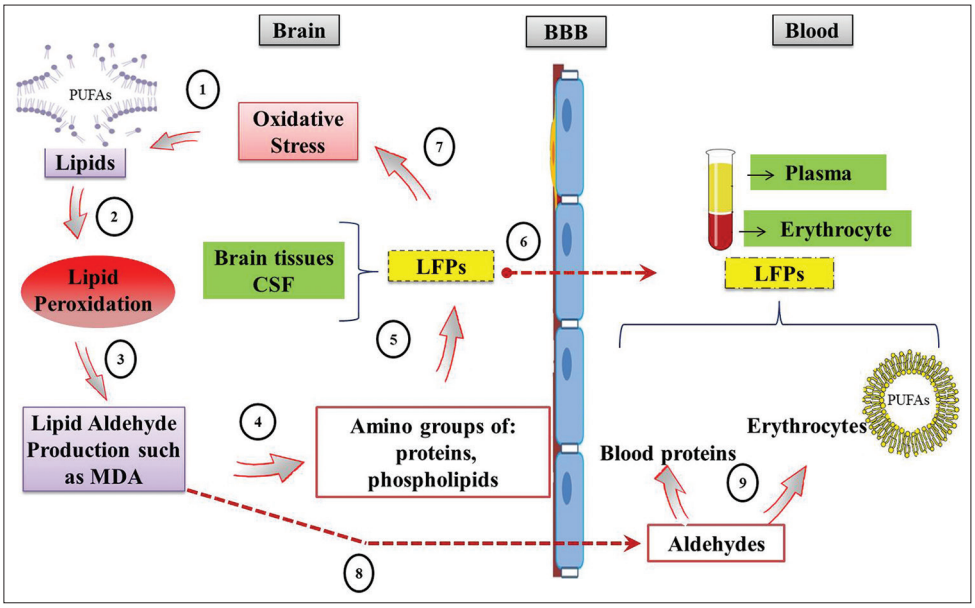

Figure 2: The origin of lipophilic fluorescent products (LFPs) in the brain (central) and blood (peripheral). An increase in the production of free radicals in the brain (1) causes the lipid peroxidation process (2). Mediators resulting from lipid peroxidation such as aldehydes (3) react with amino groups of proteins, phospholipids and nucleic acids (4) and produce LFPs (5). The LFPs formed in the brain can be secreted to the cerebrospinal fluid and the other hands are small and lipophilic intermediates that can spread across the blood–brain barrier to the blood (6). Accumulation of these compounds causes more reactive oxygen species production and increases the sensitivity of cells to oxidative damage (7). Reactive intermediates of lipid peroxidation can diffuse to the blood where they can be detected (8) and attack erythrocyte polyunsaturated fatty acids and plasma proteins and resulting in the generation of LFPs in the blood (9). LFPs = Lipophilic fluorescent products; BBB = Blood–brain barrier

Table 1: The animal and clinical studies that have analyzed the lipophilic fluorescent products as an indicator of oxidative stress in relation to brain diseases (sorted by year of research, newest to oldest)

Model of disease	Tissue target	Changes in LFPs	References
Alzheimer's disease model in rats after intracerebroventricular injection of amyloid beta 25–35	Hippocampus, CSF, plasma, and erythrocyte	Increase	[14]
Alzheimer's disease patients and mild cognitive impairment	Plasma	Increase	[65]
Alzheimer's disease patients	Erythrocytes, plasma	Increase	[62]
Alzheimer's disease patients	Erythrocytes	Increase	[63]
Alzheimer's disease patients	Erythrocytes	Increase	[64]
Alzheimer's disease patients	Erythrocytes	Increase	[38]
Multiple sclerosis's disease patients	Plasma	Increase	[66]
Canine with Alzheimer-like pathology	Brain	Increase	[67]
Canine Alzheimer's disease model	Erythrocytes	Increase	[68]

LFPs=Lipophilic fluorescent products; CSF=Cerebrospinal fluid

Table 2: The animal and clinical studies that have analyzed the lipophilic fluorescent products as an indicator of oxidative stress in relation to other diseases (sorted by year of research, newest to oldest)

Model of disease	Tissue target	Changes in LFPs	References
Intraperitoneal injection of green synthesized silver nanoparticles in rats	Hippocampus, liver, kidney, and spleen	Increase	[24]
Intracerebroventricular injection of green synthesized silver nanoparticles in rats	Hippocampus	Increase	[25]
In vitro incubation of rat brain homogenates with lead acetate	Brain	Increase	[69]
Hypoxia model in male rats	Erythrocytes	Increase	[70]
Men with coronary heart disease	Plasma	Increase	[49]
Patients with coronary heart disease	Plasma	Increase	[49]
Patients with coronary heart disease	Plasma	Increase	[40]
Ischemia/reperfusion injury in pregnant rats	Cardiac	Increase	[71]
Ischemia/reperfusion and hyperoxia model on rats	Retina	Increase	[72]
Hypoxia model on rats	Spleen, erythrocytes	Increase	[39]
Physical exercise in man	Serum	Not change	[73]
Ionizing irradiation in rats	Spleen, erythrocytes	Increase	[74]
Gamma irradiation in rats	Epididymal adipose tissue	Increase	[75]
Gamma irradiation in rats	Adipose tissue	Increase	[76]
Ionizing irradiation in rats	Mitochondria	Increase	[77]
Gamma irradiation in rats	Liver	Increase	[78]
Patients treated with diaminodiphenyl sulfone	Erythrocytes	Increase	[79]

LFPs=Lipophilic fluorescent products

However, the advantage of using LFPs as markers is that their fluorescence properties, which indicate their composition, are specific to different pathologies and can be used as fingerprints for a specific disease. Although more extensive research is needed to introduce LFPs as a diagnostic marker for brain damage. In fact, fluorescent products with high potential diagnostic value can be used as diagnostic markers in brain damage.

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Conflicts of interest
There are no conflicts of interest.

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