

IMPACT OF LIPID PEROXIDATION ON THE PHYSIOLOGY AND PATHOPHYSIOLOGY OF CELL MEMBRANES

EDITED BY : Angel Catalá and Mario Díaz
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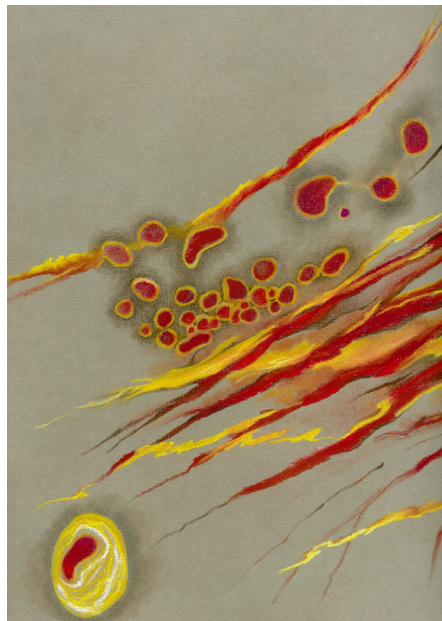
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IMPACT OF LIPID PEROXIDATION ON THE PHYSIOLOGY AND PATHOPHYSIOLOGY OF CELL MEMBRANES

Topic Editors:

Angel Catalá, Universidad Nacional de La Plata, Argentina

Mario Díaz, Universidad de La Laguna & Unidad Asociada de Investigación CSIC-ULL “Fisiología y Biofísica de la Membrana Celular en Patologías Neurodegenerativas y Tumorales”, Spain



Abstraction on the generation of oxidative stress and senile plaques and neurofibrillary tangles in the hippocampus of Alzheimer's disease brains.

Painted by Mario Díaz (2006)

The general process of lipid peroxidation consists of three stages: initiation, propagation, and termination. The initiation phase of lipid peroxidation includes hydrogen atom abstraction. Several species can abstract the first hydrogen atom and include the radicals: hydroxyl, alkoxyl, peroxy, and possibly HO^{\bullet} . The membrane lipids, mainly phospholipids, containing polyunsaturated fatty acids are predominantly susceptible to peroxidation because abstraction from a methylene group of a hydrogen atom, which contains only one electron, leaves at the back an unpaired electron on the carbon. The initial reaction of $^{\bullet}\text{OH}$ with polyunsaturated fatty acids produces a lipid radical (L^{\bullet}), which in turn reacts with molecular oxygen to form a lipid hydroperoxide

(LOOH). Further, the LOOH formed can suffer reductive cleavage by reduced metals, such as Fe^{++} , producing lipid alkoxyl radical (LO^*). Peroxidation of lipids can disturb the assembly of the membrane, causing changes in fluidity and permeability, alterations of ion transport and inhibition of metabolic processes. In addition, LOOH can break down, frequently in the presence of reduced metals or ascorbate, to reactive aldehyde products, including malondialdehyde (MDA), 4-hydroxy-2-nonenal (HNE), 4-hydroxy-2-hexenal (4-HHE) and acrolein. Lipid peroxidation is one of the major outcomes of free radical-mediated injury to tissue mainly because it can greatly alter the physicochemical properties of membrane lipid bilayers, resulting in severe cellular dysfunction. In addition, a variety of lipid by-products are produced as a consequence of lipid peroxidation, some of which can exert beneficial biological effects under normal physiological conditions. Intensive research performed over the last decades have also revealed that by-products of lipid peroxidation are also involved in cellular signalling and transduction pathways under physiological conditions, and regulate a variety of cellular functions, including normal aging. In the present collection of articles, both aspects (adverse and beneficial) of lipid peroxidation are illustrated in different biological paradigms. We expect this eBook may encourage readers to expand the current knowledge on the complexity of physiological and pathophysiological roles of lipid peroxidation.

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Table of Contents

05 Editorial: Impact of Lipid Peroxidation on the Physiology and Pathophysiology of Cell Membranes

Angel Catalá and Mario Díaz

Section 1: Lipid peroxidation and membrane pathology

08 Lipid peroxidation: pathophysiological and pharmacological implications in the eye

Ya Fatou Njie-Mbye, Madhura Kulkarni-Chitnis, Catherine A. Opere, Aaron Barrett and Sunny E. Ohia

18 Lipid peroxidation modifies the assembly of biological membranes “The Lipid Whisker Model”

Ángel Catalá

22 Interaction of aldehydes derived from lipid peroxidation and membrane proteins

Stefania Pizzimenti, Eric Ciamporcerio, Martina Daga, Piergiorgio Pettazzoni, Alessia Arcaro, Gianpaolo Cetrangolo, Rosalba Minelli, Chiara Dianzani, Alessio Lepore, Fabrizio Gentile and Giuseppina Barrera

39 Nitric oxide and membrane lipid peroxidation in photosynthetic and non-photosynthetic organisms under several stress conditions

Andrea Galatro, Paula M. González, Gabriela Malanga, Elizabeth Robello, Natacha E. Piloni and Susana Puntarulo

42 Two dimensional blue native/SDS-PAGE to identify mitochondrial complex I subunits modified by 4-hydroxynonenal (HNE)

Jinzi Wu, Xiaoting Luo and Liang-Jun Yan

Section 2. Membrane unsaturation, lipid peroxidation and cell physiology

49 Membrane lipid unsaturation as physiological adaptation to animal longevity

Alba Naudí, Mariona Jové, Victòria Ayala, Manuel Portero-Otín, Gustavo Barja and Reinald Pamplona

62 Melatonin reduces lipid peroxidation and membrane viscosity

Russel J. Reiter, Dun-Xian Tan and Annia Galano

66 Docosahexaenoic (DHA) modulates phospholipid-hydroperoxide glutathione peroxidase (Gpx4) gene expression to ensure self-protection from oxidative damage in hippocampal cells

Verónica Casañas-Sánchez, José A. Pérez, Noemí Fabelo, David Quinto-Aleman and Mario L. Díaz

77 Sub-toxic Ethanol Exposure Modulates Gene Expression and Enzyme Activity of Antioxidant Systems to Provide Neuroprotection in Hippocampal HT22 Cells

Verónica Casañas-Sánchez, José A. Pérez, David Quinto-Aleman and Mario Díaz



Nitric oxide and membrane lipid peroxidation in photosynthetic and non-photosynthetic organisms under several stress conditions

Andrea Galatro, Paula M. González, Gabriela Malanga, Elizabeth Robello, Natacha E. Piloni and Susana Puntarulo*

Physical Chemistry, School of Pharmacy and Biochemistry, Institute of Biochemistry and Molecular Medicine, University of Buenos Aires-CONICET, Buenos Aires, Argentina

*Correspondence: susanap@ffyb.uba.ar

Edited by:

Angel Catala, Universidad Nacional de La Plata, Argentina

Reviewed by:

Alexi Alekov, Medizinische Hochschule Hannover, Germany

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INTRODUCTION

Oxidative damage to lipids was characterized in terms of the nature of the oxidant, the type of lipid, and the severity of the oxidation (Simontacchi et al., 2011). Even though malondialdehyde detection with the thiobarbituric acid reactive substances test (TBARS) is the most currently used assay for the determination of lipid oxidation, it is unspecific since the reaction can be reproduced by other biological compounds (Simontacchi et al., 2011). On the other hand, electron paramagnetic resonance (EPR) spectroscopy showed the capacity of detecting the presence of the lipid radicals (LR•) formed during peroxidation, by yielding unique and stable products with spin traps (Malanga and Puntarulo, 2012). Nitric oxide (NO) is recognized both, as a signaling molecule that regulates many enzyme activities, but as a toxic agent as well. It has been found that NO is able to protect animal and plant cell types from oxidative damage resulting from superoxide (O_2^-), hydrogen peroxide (H_2O_2) and alkyl peroxides by acting as a terminator of free radical chain reactions (Wink et al., 1995, 1996; Yalowich et al., 1999; Beligni and Lamattina, 2002; Sharpe et al., 2003). Reactive oxygen species (ROS) and reactive nitrogen species (RNS) interact through the reaction of O_2^- with NO, to generate peroxynitrite ($ONOO^-$) at a rate close to diffusion. $ONOO^-$ acts as both, a nitrating agent and a powerful oxidant capable of modifying proteins (formation of nitrotyrosine), lipids (lipid oxidation, lipid nitration), and nucleic acids (DNA

oxidation and DNA nitration) (Gisone et al., 2004).

The purpose of this commentary is to point out that NO complex interactions with other cellular components lead to a wide range of effects depending on the biological system under study and the oxidative stress condition.

LIPID PEROXIDATION AND NO IN PHOTOSYNTHETIC ORGANISMS

In cultures of the green algae *Chlorella vulgaris* no significant changes were observed in either of the parameters showed in Table 1, in the stationary phase as compared to the log phase of growth. However, Qian et al. (2009) demonstrated in *Chlorella vulgaris* that, depending on its concentration, NO increased the activity of antioxidant enzymes to protect against the oxidative damage caused by herbicide stress. Data from Simontacchi et al. (2004) showed that the NO steady state concentration in homogenates from sorghum embryonic axes reached a maximum at 24–30 h after the starting of imbibition, in coincidence with the initiation of an active germination. The generation of LR• in sorghum axes was drastically increased at 36 h of imbibition from non-detectable values at 24 h, coincidentally with the significant decline in the content of cellular NO (Table 1).

To analyze the role of NO during senescence, Jasid et al. (2009) sprayed daily soybean cotyledons with an NO donor solution (sodium nitroprusside, SNP), from day 5 to 25. Differently from naturally senescent cotyledons, where NO

content reached a maximum at day 10 of seedling development and declined, in SNP-treated cotyledons NO content was higher, and remained unchanged from day 10 to 25 after germination, as compared to control values. While naturally senescent cotyledons experimented no change in the content of LR• during the studied period, SNP-treated cotyledons showed a decrease as time progressed (Table 1).

Shi et al. (2005) suggested that the protective effect of NO on the oxidative damage of thylakoid membrane proteins in *Phaseolus vulgaris* beans under UV-B radiation may be mediated by increasing the level of expression of genes encoding ROS-scavenging enzymes. Moreover, Jasid et al. (2006) showed that isolated chloroplasts from soybean leaves exposed to 2 μ M NO for 30 min, decreased the rate of LR• generation (Table 1). Also, Jasid et al. (2008) reported that axes from sorghum seeds incubated 24 h in the presence of SNP, showed a significantly higher NO content as compared to control axes, and LR• content evaluated in the microsomal fractions was significantly lower, as compared to control membranes (Table 1).

LIPID PEROXIDATION AND NO IN NON-PHOTOSYNTHETIC ORGANISMS

Data in Table 1 show that seasonality affects oxidative metabolism in digestive glands (DG) from *Nacella magellanica* limpets isolated from the Beagle Channel, Tierra del Fuego, Argentina. A significant increase in both LR• and NO content in summer, as compared to winter,

Table 1 | Nitric oxide and lipid peroxidation under stress conditions in photosynthetic and non-photosynthetic organisms.

Biological system	Stress condition	Lipid peroxidation		NO	References
		TBARS	LR•		
PHOTOSYNTHETIC ORGANISMS					
Intact cells					
Chlorella cells	Development	No change day 12–18	No change day 12–18	No change day 12–18	Malanga and Puntarulo, 1995; Estevez et al., 2001; Estévez and Puntarulo, 2005
Homogenates					
Sorghum embryonic axes	Development	nd	Increased from 36 to 48	Decreased from 36 to 48 h	Simontachi et al., 2004
Soybean cotyledons	Senescence	nd	No change day 10–25	Non-detectable at day 25	Jasid et al., 2009
	+SNP	nd	Decrease 58% day 10–25	No change day 10–25	
Sub-cellular structures					
Chloroplasts from soybean leaves	+GSNO 250 μM	nd	Decrease 29%	2 μM NO (supplementation)	Jasid et al., 2006
Microsomes from sorghum embryonic axes	+SNP 1 mM	nd	Decrease 43%	Increase 140%	Jasid et al., 2008
NON-PHOTOSYNTHETIC ORGANISMS					
Invertebrates					
Nacella magellanica	Summer vs. winter	nd	1.7-fold increase	1.6-fold increase	Malanga et al., 2007
Mya arenaria	Fe 500 μM	4-fold increase day 0–17	nd	4-fold decrease day 0–17	González et al., 2010
Mammals					
Fetus rat brain	γ radiation 2 h	No change	No change	6-fold increase	Gisone et al., 2003
	γ radiation 4 h	Increase 51%	nd	No change	Gisone et al., 2003
Rat liver	Fe 500 mg/kg	2.7-fold increase	nd	No change	Galleano and Puntarulo, 1992; Rousseau et al., 2011

nd stands for non-determined.

was reported (Malanga et al., 2007). However, studies on toxicological effects of Fe exposure under laboratory conditions showed that significant increases in lipid peroxidation were temporarily associated to decreases in NO content in DG from the bivalve *Mya arenaria* after 17 days of treatment (González et al., 2010) (Table 1).

In vivo γ irradiation of rat fetuses did not significantly affect neither the content of LR* nor the content of TBARS in the brain up to 2 h post-irradiation (pi). However, 4 h after the exposure, a significant increase in the TBARS content was measured. These results are consistent with the hypothesis that changes could be produced in the brain at the early stages after exposure to γ radiation to limit free radical-dependent damage, since increased lipid peroxidation was only

detected after 4 h pi. Gisone et al. (2003) showed that total NO synthase activity was increased after 30 and 60 min pi, and returned to control values after 2 h pi, and accordingly NO content was significantly increased (Table 1).

Galleano and Puntarulo (1992) showed that liver homogenates from Fe-dextran overloaded male Wistar rats showed a significant increase in TBARS 6 h post-injection, as compared to control rats (Table 1). Later, Galleano et al. (2001) pointed out that the significant increase in NO, assayed as DETC₂-Fe-NO adducts 5 h after Fe administration could be an artifact due to the excess of Fe during the measurement. Recently, Rousseau et al. (2011) showed that one of the molecular footprints left by the reactions of ROS with biomolecules, the level of protein 3-nitrotyrosines, was not increased by

Fe-dextran administration, suggesting that Fe overload in liver did not change NO cellular content (Table 1).

CONCLUDING REMARKS

The results summarized here implied the existence of a very complex regulatory interplay between NO and ROS. The multiple effects of NO on the process of lipid peroxidation imply that the net result will depend on the balance of competing factors. The rate and location of NO formation, and also the rate of formation of O₂⁻, or other mitigating factors, will all contribute to the degree and the nature of the effect on lipid oxidation in a particular system. Detailed analysis of the molecular mechanisms in each condition is required. In this regard, no yet deeply studied NO reactions, such as NO binding to Fe and

endogenous thiols and other nitrosyl–Fe complexes that seems to favor Fe release from the cell avoiding its accumulation, could reveal to be a key factor in NO cellular interactions and should be further characterized.

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