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

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 (Malang 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$_{2}$O$_{2}$) 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 Chlorellavulgaris 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 Simontachi et al. (2004) showed that the NO steady state concentration, NO increased the activity of antioxidant enzymes to protect against the oxidative damage caused by herbicide stress. Data from Simontachi et al. (2004) showed that the NO steady state concentration, NO increased the activity of antioxidant enzymes to protect against the oxidative damage caused by herbicide stress. Data from Simontachi et al. (2004) showed that the NO steady state concentration, NO increased the activity of antioxidant enzymes to protect against the oxidative damage caused by herbicide stress. Data from Simontachi et al. (2004) showed that the NO steady state concentration, NO increased the activity of antioxidant enzymes to protect against the oxidative damage caused by herbicide stress. Data from Simontachi et al. (2004) showed that the NO steady state concentration, NO increased the activity of antioxidant enzymes to protect against the oxidative damage caused by herbicide stress. Data from Simontachi et al. (2004) showed that the NO steady state concentration, NO increased the activity of antioxidant enzymes to protect against the oxidative damage caused by herbicide stress. Data from Simontachi et al. (2004) showed that the NO steady state concentration, NO increased the activity of antioxidant enzymes to protect against the oxidative damage caused by herbicide stress. Data from Simontachi et al. (2004) showed that the NO steady state concentration, NO increased the activity of antioxidant enzymes to protect against the oxidative damage caused by herbicide stress. Data from Simontachi et al. (2004) showed that the NO steady state concentration, NO increased the activity of antioxidant enzymes to protect against the oxidative damage caused by herbicide stress. Data from Simontachi et al. (2004) showed that the NO steady state concentration, NO increased the activity of antioxidant enzymes to protect against the oxidative damage caused by herbicide stress. Data from Simontachi et al. (2004) showed that the NO steady state concentration, NO increased the activity of antioxidant enzymes to protect against the oxidative damage caused by herbicide stress. Data from Simontachi et al. (2004) showed that the NO steady state concentration, NO increased the activity of antioxidant enzymes to protect against the oxidative damage caused by herbicide stress. Data from Simontachi et al. (2004) showed that the NO steady state concentration, NO increased the activity of antioxidant enzymes to protect against the oxidative damage caused by herbicide stress. Data from Simontachi et al. (2004) showed that the NO steady state concentration, NO increased the activity of antioxidant enzymes to protect against the oxidative damage caused by herbicide stress. Data from Simontachi et al. (2004) showed that the NO steady state concentration, NO increased the activity of antioxidant enzymes to protect against the oxidative damage caused by herbicide stress. Data from Simontachi et al. (2004) showed that the NO steady state concentration, NO increased the activity of antioxidant enzymes to protect against the oxidative damage caused by herbicide stress. Data from Simontachi et al. (2004) showed that the NO steady state concentration, NO increased the activity of antioxidant enzymes to protect against the oxidative damage caused by herbicide stress. Data from Simontachi et al. (2004) showed that the NO steady state concentration, NO increased the activity of antioxidant enzymes to protect against the oxidative damage caused by herbicide stress. Data from Simontachi et al. (2004) showed that the NO steady state concentration, NO increased the activity of antioxidant enzymes to protect against the oxidative damage caused by herbicide stress.

Keywords: nitric oxide, lipid peroxidation, photosynthetic organisms, animals, oxidative stress conditions
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       | Development      | No change day 12–18| No change day 12–18 | Malanga and Puntarulo, 1995; Estevez et al., 2001; Estévez and Puntarulo, 2005 |
| Chlorella cells    | Development      | No change day 12–18| No change day 12–18 | Simontachi et al., 2004 |
| Homogenates        | Development      | Increased from 36 to 48 | Decreased from 36 to 48 h | Jasid et al., 2009 |
| Soybean cotyledons | Senescence       | No change day 10–25| Non-detectable at day 25 | Jasid et al., 2006 |
| +SNP               | nd               | Decrease 58% day 10–25 | No change day 10–25 | Jasid et al., 2008 |
| Sub-cellular structures |                |                    |    |                                                 |
| Chloroplasts from soybean leaves | +GSNO 250 μM | No change | Decrease 3% | 2 μM NO (supplementation) |
| Microsomes from sorghum embryonic axes | +SNP 1 mM | No change | Decrease 44% | Increase 140% |
| **NON-PHOTOSYNTHETIC ORGANISMS** |                  |                    |    |                                                 |
| Invertebrates      |                  |                    |    |                                                 |
| Nacella magellanica | Summer vs. winter | 4-fold increase day 0–17 | 1.7-fold increase | Malanga et al., 2007 |
| Mya arenaria       | Fe 500 μM        | nd                 | nd  | Gonzalez et al., 2010 |
| Mammals            |                  |                    |    |                                                 |
| Fetus rat brain    | γ radiation 2 h  | No change          | No change | Gisone et al., 2003 |
|                    | γ radiation 4 h  | Increase 1%         | No change | Gisone et al., 2003 |
| Rat liver          | Fe 500 mg/kg     | 2.7-fold increase   | nd  | 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 temporally 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 thiol 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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REFERENCES
Beligni, M. V., and Lamattina, L. (2002). Nitric oxide interferes with plant photo-oxidative stress by detoxifying reactive oxygen species. *Plant Cell Environ.* 25, 737–748. doi: 10.1046/j.1365-3040.2002.00857.x
Estevez, M. S., Malanga, G., and Puntarulo, S. (2001). Iron-dependent oxidative stress in *Chlorella vulgaris*. *Plant Sci.* 161, 9–17. doi: 10.1016/S0168-9452(01)00364-8
Estevez, M., and Puntarulo, S. (2005). Nitric oxide generation upon growth of Antarctic *Chlorella* sp. cells. *Physiol. Plantarum* 125, 192–201. doi: 10.1111/j.1399-3054.2005.00561.x
Galleano, M., Aimo, L., Borroni, M. V., and Puntarulo, S. (2001). Nitric oxide and iron overload. Limitations or ESR detection by DETC. *Toxicology* 167, 199–205. doi: 10.1016/S0300-483X(01)00474-7
Galleano, M., and Puntarulo, S. (1992). Hepatic chemiluminescence and lipid peroxidation in mild iron overload. *Toxicology* 76, 27–38. doi: 10.1016/0300-483X(92)90015-7
Gisone, P., Boveris, A. D., Dubner, D., Perez, M. R., Robello, E., and Puntarulo, S. (2003). Early neuroprotective effect of nitric oxide in developing rat brain irradiated in utero. *Neurotoxicology* 24, 245–253. doi: 10.1016/S0161-813X(02)00166-3
Gisone, P., Dubner, D., Perez, M. R., Michelin, S., and Puntarulo, S. (2004). The role of nitric oxide in the radiation-induced effects in the developing brain. *In Vivo* 18, 281–292.
González, P. M., Abele, D., and Puntarulo, S. (2010). Exposure to excess dissolved iron in vivo affects oxidative status in the bivalve *Mya arenaria*. *Comp. Biochem. Physiol. C* 152, 167–174. doi: 10.1016/j.cbpc.2010.04.006
Jasid, S., Galatro, A., Villordo, J. I., Puntarulo, S., and Simontacchi, M. (2009). Role of nitric oxide in soybean cotyledon senescence. *Plant Sci.* 176, 662–668. doi: 10.1016/j.plantsci.2009.02.007
Jasid, S., Simontacchi, M., Bartoli, C. G., and Puntarulo, S. (2006). Chloroplasts as a nitric oxide cellular source. Effect of reactive nitrogen species on chloroplastic lipids and proteins. *Plant Physiol.* 142, 1246–1255. doi: 10.1104/pp.106.086918
Jasid, S., Simontacchi, M., and Puntarulo, S. (2008). Exposure to nitric oxide protects against oxidative damage but increases the labile iron pool in sorghum embryonic axes. *J. Exp. Bot.* 59, 3953–3962. doi: 10.1093/jdb/ern235
Malanga, G., Estevez, S., Calvo, J., Abele, D., and Puntarulo, S. (2007). Seasonality effect on oxidative metabolism in *Nasella (P.) magellanicus*. *Comp. Biochem. Physiol. A* 146, 551–558. doi: 10.1016/j.cbpa.2006.01.029
Malanga, G., and Puntarulo, S. (1995). Oxidative stress and antioxidant content in *Chlorella vulgaris* after exposure to ultraviolet-B radiation. *Physiol. Plantarum* 94, 672–679. doi: 10.1111/j.1399-3054.1995.tb00983.x
Malanga, G., and Puntarulo, S. (2012). “The use of electron paramagnetic resonance (EPR) in the study of oxidative damage to lipids in aquatic ecosystems,” in Book on Oxidative Stress in Aquatic Ecosystems, eds D. Abele, T. Zenteno-Savin, and J. P. Vázquez-Medina (Oxford, UK: Willey-Blackwell), 448–457.
Qian, H., Chen, W., Li, J., Wang, J., Zhou, Z., Liu, W., et al. (2009). The effect of exogenous nitric oxide on alleviating herbicide damage in *Chlorella vulgaris*. *Aquat. Toxicol.* 92, 250–257. doi: 10.1016/j.aquatox.2009.02.008
Rousseau, I., Galleano, M., and Puntarulo, S. (2011). Fe allocation in liver during early stages of endotoxemia in Fe-overload rats. *Toxicol. Pathol.* 39, 1075–1083. doi: 10.1177/0192623311425057
Sharpe, M. A., Robb, S. J., and Clark, J. B. (2003). Nitric oxide and Fenton/Haber–Weiss chemistry: nitric oxide is a potent antioxidant at physiological concentrations. *J. Neurochem.* 87, 386–394. doi: 10.1046/j.1471-4159.2003.02001.x
Shi, S., Wang, G., Wang, Y., Zhang, L., and Zhang, L. (2005). Protective effect of nitric oxide against oxidative stress under ultraviolet-B radiation. *Nitric Oxide* 13, 1–9. doi: 10.1016/j.niox.2005.04.006
Simontacchi, M., Buett, A., and Puntarulo, S. (2011). “The use of electron paramagnetic resonance (EPR) in the study of oxidative damage to lipids in plants,” in Lipid Peroxidation: Biological Implications, ed A. Catalá (Kerala, India: Res. Signpost Transworld Res. Network), 141–160. ISBN: 978-81-7895-527-8
Simontacchi, M., Jasid, S., and Puntarulo, S. (2004). Nitric oxide generation during early germination of sorghum seeds. *Plant Sci.* 167, 839–847. doi: 10.1016/j.plantsci.2004.05.028
Wink, D. A., Cook, J. A., Pacelli, R., Degraffi, W., Gamson, J., Liebmann, J., et al. (1996). The effect of various nitric oxide-donor agents on hydrogen peroxide-mediated toxicity: a direct correlation between nitric oxide formation and protection. *Arch. Biochem. Biophys.* 331, 241–248. doi: 10.1006/abbi.1996.0304
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