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M. BALAZY

TRANS-ARACHIDONIC ACIDS: NEW MEDIATORS OF INFLAMMATION

New York Medical College, Department of Pharmacology, Valhalla, NY 10595 USA

Inflammation and many other pathological processes lead to increased production of free radicals that target critical macromolecules such as proteins, DNA and lipids. Structural modifications of these molecules, induced by free radicals, typically result in alterations of vital biochemical processes. Hydroxyl radical-initiated lipid peroxidation is known to generate a variety of toxic oxidized lipids, many of which originate from polyunsaturated fatty acids esterified to cellular membrane phospholipids. Recent interests have focused on a group of lipids known as isoeicosanoids that are formed from peroxidation of arachidonic acid, and share structural similarity to enzymatically-derived prostaglandins and leukotrienes. However, little is known about lipid peroxidation processes initiated by nitrogen free radicals. NO_2 is a toxic free radical and an abundant urban air pollutant, which is also generated *in vivo* from oxidations of nitric or nitrite and decomposition of peroxynitrite. The NO_2-induced lipid peroxidation mechanisms involving arachidonic acid have not been characterized. Described here is the isomerization of arachidonic acid, a new process induced by NO_2, which leads to a mixture of *trans*-arachidonic acids. We observed that the levels of *trans*-arachidonic acids in rat plasma increased following infusion of bacterial endotoxin; therefore, the isomerization of arachidonic acid is likely to occur *in vivo* by a mechanism involving NO_2.

Key words: *free radicals, trans-arachidonic acid, isoeicosanoids, NO_2-induced lipid peroxidation, uric acid.*

INTRODUCTION

Trans fatty acids

Trans fatty acids are those unsaturated fatty acids that have one or more *trans* double bonds instead of *cis* bonds. *Trans* fatty acids are found in dairy fats and, to a greater extent, in products made from hydrogenated fat, such as margarine. Typical Western diets contain an estimated 5—6% of *trans* fatty acids (as percentage of total fat intake). Despite numerous epidemiological and

experimental feeding studies, evidence regarding the health risk of *risk* of *trans* fatty acids is not conclusive (1—4). It is known that increased levels of *trans* fatty acids can alter biological membrane rigidity, permeability and asymmetry (5—7). It has been also noted that one of the serious limitations toward understanding the potential role of *trans* fatty acids as causative factors in the development of cardiovascular disease is the lack of a precise and accurate methods for their quantitative analysis (1). In addition, it has been known that *trans* fatty acids are not a homogenous group and individual *trans* isomers may have a different biological profile (1).

We obtained evidence that *trans* fatty acids are formed within biological membranes phospholipids by a free radical process initiated by NO_2 (8). Because NO_2 is a product of NO oxidation, the isomerization of unsaturated fatty acids is likely to occur *in vivo*, particularly under conditions such as inflammation, which causes induction of NO biosynthesis. Therefore, *trans* fatty acids may originate not only from the diet but also from a reaction between precursor-membrane bound fatty acids and NO_2. We focused our studies on the isomerization of arachidonic acid, which is an aboundant fatty acid esterified to cellulalr membrane phospholipids, and a precursor for important lipid mediators. Our studies revealed that the isomerization of arachidonic acid is a specific and characteristic process of NO_2, OH, peroxyl, and superoxide do not isomerize arachidonic acid (8). The isomerization of arachidonic acid has also been observed following exposure of cells to peroxynitrite, and an involvement of NO_2 in this process has been suggested (9). Thus, endogenous formation of *trans* fatty acids, such as *trans*-arachidonic acids could be used as a marker of the exposure of biological membranes to NO_2.

Nitrogen dioxide and lipid peroxidation

NO_2 is a major toxic air pollutant that originates from the combustion of fossil fuels. Therefore, available studies have exclusively focused on the toxicity of inhaled NO_2 (10—12). Changes in cellular membrane asymmetry and fluidity have been observed following exposure to NO_2 (7, 13). NO_2 can be also generated endogenously by several mechanisms involving oxidation of NO or nitrite (Table 1). However, the potential of endogenously formed NO_2 to induce lipid peroxidation remains to be explored. Liu *et al.* have observed that oxidation of NO to NO_2 in aqueous solutions is greatly accelerated by the addition of phospholipids and the NO oxidation occurs predominantly in the hydrophobic bilayer of phospholipid vessicles (21). This study indicates that the biological membrane may play an important role in the oxidative chemistry of NO. In the lipid phase, the hydrolysis of NO_2 to nitrite and nitrate is minimal (22). Thus, NO_2 may remain in membrane lipids for periods of time that may

be sufficient for reaction with fatty acids and phospholipids. Reaction of NO_2 with another NO molecule produces dinitrogen trioxide (N_3O_3), a potent nitrosating agent. In the aqueous phase, N_2O_3 rapidly hydrolyzes to form two NO_2^- molecules. NO_2^- can be oxidized to NO_2 enzymatically in the presence of H_2O_2 (reactions 3—6 and 8 in *Table 1*). Thus, some of NO_2^- may be converted to NO_2. The rate of reaction 1 (*Table 1*) is 10^4-fold slower than reaction 2 indicating that at constant concentrations of NO, oxygen and superoxide, the majority of NO will react with superoxide to form peroxynitrite ($ONOO^-$). $ONOO^-$ is protonated in biological buffers to peroxynitrous acid, which decomposes to NO_2 and a molecule of OH-radical reactivity.

Table 1. Mechanisms of NO_2 generation

Reaction	Reference
1. $2NO + O_2 \rightarrow 2NO_2$	(14)
2. $NO + O_2 \rightarrow ONOO^- + H^+ \rightarrow ONOOH \rightarrow NO_2 + OH$	(14)
3. myeloperoxidase + H_2O_2 + nitrite $\rightarrow NO_2$	(15)
4. myeloperoxidase + H_2O_2 + Cl^- + nitrite $\rightarrow NO_2$	(16)
5. Cu,Zn-SOD + H_2O_2 + nitrite $\rightarrow NO_2$	(17)
6. peroxidases + H_2O_2 + nitrite $\rightarrow NO_2$	(18)
7. cytochrome P450 + $ONOO^- \rightarrow NO_2$	(19)
8. lactoperoxidase + H_2O_2 + nitrite $\rightarrow NO_2$	(20)

Prütz *et al.* have shown that exposure of arachidonic acid to NO_2 in aqueous solutions generates arachidonyl radicals with a rate of $\approx 10^6\ M^{-1}s^{-1}$ (23). Therefore, the reaction of NO_2 with arachidonic acid occurs faster than the disproportionation of NO_2 to nitrite and nitrate (23). NO_2-mediated autooxidation of unsaturated lipids is not autocatalytic and is not dependent on basal hydroperoxides. These two aspects differentiate NO_2-mediated lipid oxidation from copper-and hydroperoxide-dependent oxidations.

Several investigators have described NO_2-initiated peroxidation of linoleic and linolenic acids ($k \approx 10^5$—$10^6 M^{-1}s^{-1}$) (24—27). In fact, the potential of nitrite to modify unsaturated fatty acids has been first described more than 180 years ago (28). Peroxynitrite has also been found to oxidize linoleic acid, producing nitro-, nitrito- and nitrosoperoxo linoleic acid products (29, 30). Reactions of linoleate hydroperoxides with HONO have produced epoxy-nitrolinoleic acids (25). Lai and Finlayson-Pitts have shown that NO_2 reacts with oleic acid bound to phospholipids (31). Infrared spectroscopy and FAB mass spectrometry revealed that nitration occurs at the double bond of the fatty acid (31). However, formation of these lipid products *in vivo* has not been reported. The major difficulty to understanding the role of NO_2 in the lipid peroxidation processes has been the lack of structural identification of the specific NO_2-derived lipid peroxidation products. We hypothesized that

characterization of the products of the NO_2-arachidonic acid reaction may yield important clues regarding novel mechanisms involved in their formation.

Arachidonic acid and lipid peroxidation

Arachidonic acid esterified to glycerophospholipids that make the cellular membrane serves as the primary precursor of a number of complex products formed by chemical reactions taking place within the biological membrane as a result of formation of reactive oxygen species. Recent studies have focused on a series of metabolites derived from arachidonic acid termed isoeicosanoids that have chemical structures similar to enzymatically-derived products. These include isoprostanes (32) and isoleukotrienes (33). These molecules have been used as markers of the initiation of free radical events within tissues by the OH radical. Other products of free radical reactions, such as malondialdehyde (MDA), 4-hydroxynonenal and pentane have been also analyzed as the marker substances. However, such low-molecular mass compounds probably originate from several reactions subsequent to the initial oxidations of a polyunsaturated fatty acid acyl group esterified to phospholipids. To characterize the initial free radical processes within cellular membranes, the more intact free radical product species may be more useful targets for structural identification. Such markers are likely to provide important clues regarding various mechanisms involved in their formation. In addition to the marker function served by these molecules, several compounds have been found to possess significant biological activity, including the isoprostanes (32) and the isoleukotrienes (33), as well as oxidatively modified phospholipids derived from membrane precursors (34). These phospholipid molecules apear to exert biological activity through specific receptors, including the PAF receptor (35).

Isomerization and nitration of arachidonic acid by NO_2

Our experiments demonstrate that arachidonic acid reacts readily with NO_2 and also with $ONOO^-$ in a dose-dependent manner, generating a complex mixture of products (8, 9, 36). An UV chromatogram obtained during analysis of the NO_2-arachidonic acid reaction mixture by a reversephase HPLC revealed major products that eluted after the peak of arachidonic acid. This was a unique finding because a product of arachidonic acid oxidation having such a chromatographic property has not been reported previously. We used various techniques of mass spectrometry, including electrospray tandem mass spectrometry to structurally characterize the new lipid products. The mass spectra revealed that the major product obtained from the NO_2-arachidonic acid reaction was a mixture of four isomers having one *trans* and three *cis* bonds (*Fig. 1*). The isomerization is likely to involve the binding of NO_2

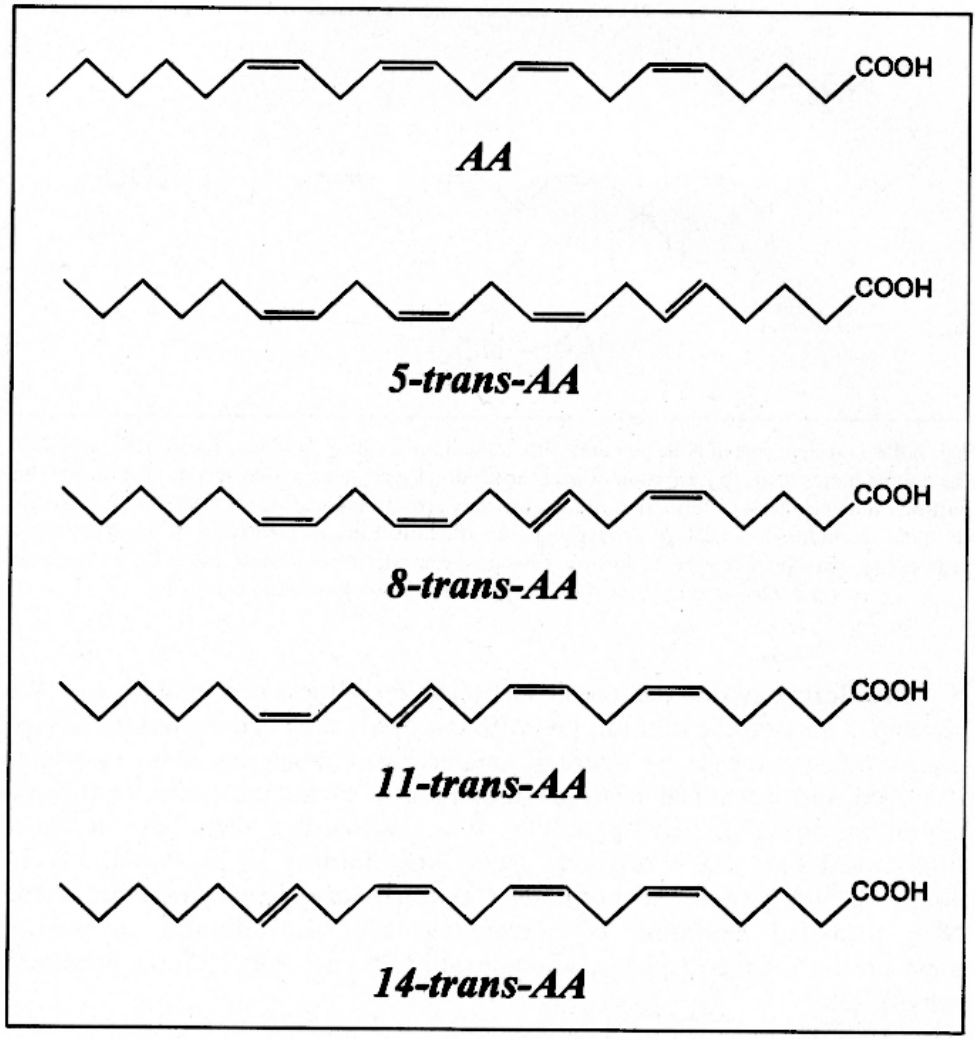

Fig. 1. Structures of arachidonic acid (AA) and four *trans*-arachidonic acids that contain one *trans*-bond and three *cis*-double bonds.

and formation of a nitroarachidonyl radical followed by elimination of NO_2 and generation of a *trans* bond. The nitroarachidonyl radical may also bind oxygen to produce nitrohydroxyeicosatrienoic acids (NO_2OHAA). The mass spectrometric analysis revealed that eight isomers of NO_2OHAA were formed (37). In addition, the NO_2/arachidonic acid reaction mixture contained nitroeicosatetraenoic acids, oxime-arachidonic acids, hydroxyeicosatetraenic acids, epoxyeicosatrienoic acids and isoprostaglandins. This study revealed that NO_2 might react with arachidonic acid by at least two mechanisms as shown in *Fig. 2.*

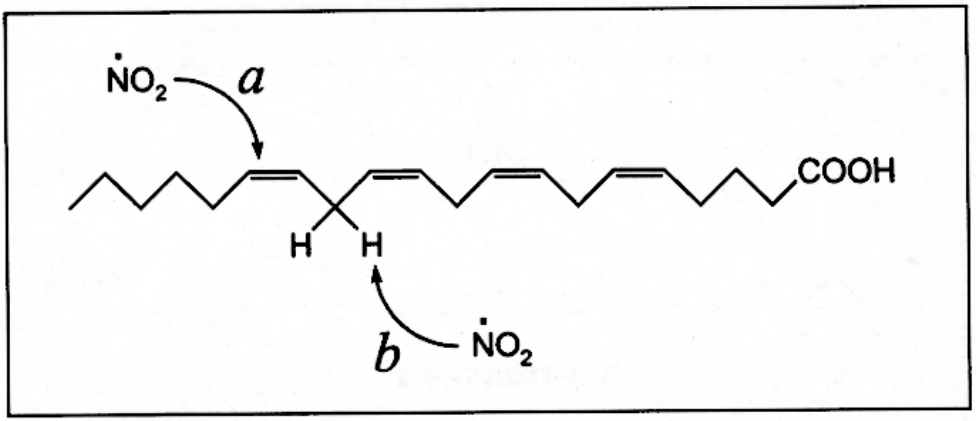

Fig. 2. Two mechanisms of NO_2 reaction with arachidonic acid. *a* — Addition separately to each of the double bonds leads to formation of *trans*-arachidonic acids *via* a nitroarachidonyl radical. This pathway may also lead to formation of nitrohydroxyeicosatrienoic acids *via* addition of oxygen to the nitroarachidonyl radical. *b* — Hydrogen abstraction leads to formation of an arachidonyl radical that may bind oxygen to form hydroperoxyeicosatetraenoic acids and other oxygenated products, or may bind NO_2 to generate nitroeicosatetraenoic acids.

Our efforts have focused on the identification of these new lipids *in vivo*. We developed an isotopic dilution GC/MS assay that uses octadeuterium-labeled *trans*-arachidonic acid as internal standard (8). Using this assay, we have identified and quantified *trans*-arachidonic acid in human plasma (50.3 ± 10 ng/ml) and urine (122 ± 50 pg/ml) (8). It is possible that these basal levels of *trans*-arachidonic acids originate from NO_2 formed by oxidation of endogenous NO. These new molecules may function as specific markers of the NO_2 mediated oxidation of arachidonic acid. In addition, several of these products have displayed vasorelaxant (37) and platelet antiaggregatory activity (38).

Endotoxemia causes increased trans-arachidonic acid levels in blood

The terms sepsis, endotoxemia, septicemia, and septic shock are used to identify the continuum of the clinical response to infection with bacterial toxins such as lipopolysaccharide (LPS). Endotoxemia is characterized by the invasion of bacterial toxins so that alterations are effected both by mediators activated by bacteria or their products and by body defense mechanisms. A substantial amount of evidence suggested the involvement of reactive oxygen species (ROS) in sepsis and inflammation, including the increase of lipid peroxidation products (16, 39, 40), the ability of ROS to mimic all facets of organ injury such as edema, hemorrhage, vascular obstruction, and impair-

ment of vessel contractility (41—45). It has also been suggested that the late phase of LPS-evoked arterial hypotension and lethal vasoplegia as well as multiple organ failure syndrome are associated with overproduction of NO (45). The role of iNOS is well documented in the pathogenesis of sepsis and endotoxemia; conditions of elevated NO levels that contribute to LPS induced hypotension and mortality.

Antioxidants such as SOD (43, 46) and catalase (47) provide partial protection during endotoxemia and show only minimal beneficial therapeutic effect (48). Immunotherapy with antibodies aimed at targeting multiple proteins has failed to improve survival from sepsis and septic shock (42). The NOS inhibitor, L-nitroarginine, administered prior to LPS, causes a dramatic increase of the mortality of rats due to acute lung injury (44). If reactive nitrogen species generated in endotoxemia cause damage to the cellular membrane, then such damage should be revealed by detection of atered lipid molecules.

We studied the effect of LPS on *trans*-arachidonic acid levels in plasma and urine in rats using the GC/MS assay (*Fig. 3*). Following infusion of LPS into the jugular vein (10 mg/kg in 10 min), the *trans*-arachidonic acid levels in plasma increased significantly, and reached concentrations of 0.5 μM after 5 hrs (*Fig. 3*). Urinary levels of these acids were not changed. Our experiments show, for the first time, that isomerization of arachidonic acid can be induced in endotoxemia. In addition, the GC/MS analysis of *trans*-arachidonic acid extracted from the blood of LPS-treated rats showed a characteristic profile that was nearly identical with the arachidonate isomer profile obtained by treatment with NO_2 (8) but different from the profile produced by the reaction of $ONOO^-$ with arachidonic acid (9). Thus it appears that NO_2 rather than $ONOO^-$ is involved in the generation of *trans*-arachidonic acids, and possibly of other fatty acids, in endotoxemia.

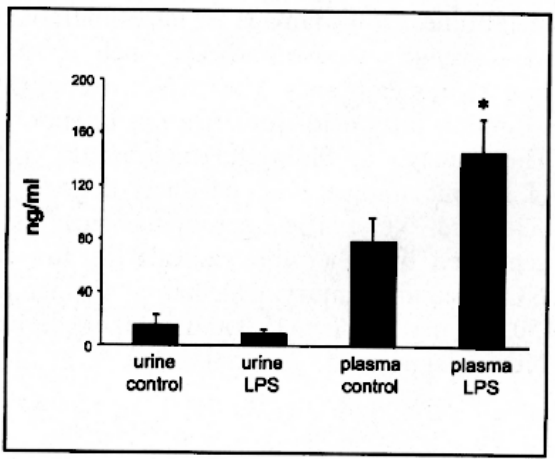

Fig. 3. Quantitative analysis of *trans*-arachidonic acids by an isotopic dilution GC/MS assay. The concentration of *trans*-arachidonic acid in plasma of LPS (serotype 0111:B4)-treated rats (10 mg/kg; 5 hrs) was 155.5 ± 33 ng/ml ($n = 6$), and was significantly higher ($p < 0.05$) than in plasma of control rats (78.6 ± 19 ng/ml, $n = 6$). Urinary levels of *trans*-arachidonic acid were 8.6 ± 3 ng/ml ($n = 6$) in LPS-treated rats and $14.8 \pm$ ng/ml ($n = 8$) in the control group.

CONCLUSIONS

The development of new methodologies based on mass spectrometry has advanced our understanding of the role of NO oxidative chemistry that takes place in biological membranes as a result of formation of active oxygen species in inflammation. The new aspect of our studies is that, for the first time, we have correlated the levels of a specific endogenous group of *trans* fatty acids with the disease process. In the past, the *trans* fatty acids were of concern as components of diet. Our data support the hypothesis that circulating *trans* fatty acids not only originate from the diet but also from the endogenous free radical processes. The accumulation of *trans* fatty acids in biological membranes is likely to alter membrane properties such as fluidity, permeability and asymmetry. Isomerization of membrane fatty acids is a newly identified process that could contribute significantly to mechanisms of free-radical-induced membrane injury. Since NO_2 has been known as a major air pollutant and has been suspected to be the cause of asthma, lung cancer (11), and cardiovascular complications (49), our observations may also advance studies of inhaled NO_2 toxicity. In addition, many researchers have noted that serious limitations in the quantitative estimation of *trans* fatty acids intake is a major problem in understanding the health effects of *trans* fatty acids. It has been also suggested that knowledge of *trans* fatty acids plasma levels in humans may be valuable in estimating the effects of particular diet regiments on health outcomes. The use of deuterium-labeled standards in the quantitative analysis has the advantage of unequivocal identification and quantification of a selected group of *trans* fatty acids. The synthesis of specific isomers of *trans*-arachidonic acid will also advance the understanding of their distribution and effects on membrane function. Our studies also suggest that scavengers of NO_2 should inhibit fatty acid isomerization and nitration, and protect biological membranes from damage in inflammation. Uric acid (UA) has the potential to scavenge reactive radicals, such as NO_2, and prevent its actions on biological membranes. The effectiveness of UA and similarly acting compounds to inhibit fatty acid isomerization in endotoxemia remains to be established. The damage to biological membranes could be assessed by measurement of specific unique lipid products derived from the reaction of arachidonic acid and NO_2. These products, such as *trans*-arachidonic acid, are not generated by other free radicals (8), thus can serve as specific markers of NO_2-mediated injury. UA has a distinct profile because it scavenges NO_2 (50, 51), peroxynitrite (52) and oxidants derived from H_2O_2 without scavenging NO or superoxide (53, 54).

REFERENCES

1. Rudrum M. Trans fatty acids: consensus and debate. *Eur J Med Res* 1995; 1: 115—117.
2. Ascherio A, Willet WC. Health effects of trans fatty acids. *Am J Clin Nutur* 1997; 66: 1006S-1010S.
3. Feldman EB, Kris-Etherton PM, Kritchevsky D, Lichtenstein AH. Position paper on trans fatty acids. *Am J Clin Nutr* 1996; 63: 663—670.
4. Nelson GJ. Dietary fat, trans fatty acids, and risk of coronary heart disease. *Nutr Rev* 1998; 56: 250—252.
5. Precht D, Molkentin J. Trans fatty acids: implications for health, analytical methods, incidence in edible fats and intake (a review). *Nahrung* 1995; 39: 343—374.
6. Mann GV. Metabolic consequences of dietary trans fatty acids. *Lancet* 1994; 343: 1268—1271.
7. Patel JM, Block ER. Nitrogen dioxide-induced changes in cell membrane fluidity and function. *Am Rev Respir Dis* 1986; 134: 1196—1202.
8. Jiang H, Kruger N, Lahiri DR, Wang D, Vatèle JM, Balazy M. Nitrogen dioxide induces cistrans isomerization of arachidonic acid within cellular phospholipids. Detection of *trans*-arachidonic acids *in vivo*. *J Biol Chem* 1999; 274: 16235—16241.
9. Boulos C, Jiang H, Balazy M. Permeability of peroxynitrite into the human platelet inhibits cyclooxygenase *via* nitration of tyrosine residues. *J Pharmacol Exp Ther* 2000; 293: 222—229.
10. Elsayed NM. Toxicity of nitrogen dioxide: an introduction. *Toxicology* 1994; 89: 161—174.
11. Environmental Health Criteria: Nitrogen Oxides. Geneva: World Health Organization, 2, 1997.
12. Postlethwait EM, Bidani A. Mechanisms of pulmonary NO_2 absorption. *Toxicology* 1994; 89: 217—237.
13. Li YD, Patel JM, Block ER. Nitrogen dioxide-induced phosphatidylserine biosynthesis and subcellular translocation in cultured pulmonary artery endothelial cells. *Toxicol Appl Pharmacol* 1994; 129: 114—120.
14. Koppenol WH. The basic chemistry of nitrogen monoxide and peroxynitrite. *Free Radic Biol Med* 1998; 25: 385—391.
15. Byun J, Mueller DM, Fabjan JS, Heinecke JW. Nitrogen dioxide radical generated by the myeloperoxidase-hydrogen peroxide-nitrite system promotes lipid peroxidation of low density lipoprotein. *FEBS Lett* 1999; 455: 243—246.
16. Eiserich JP, Hristova M, Cross CE, Jones AD, Freeman BA, Halliwell B, van der Vliet A. Formation of nitric oxide-derived inflammatory oxidants by myeloperoxidase in neutrophils. *Nature* 1998; 391: 393—397.
17. Singh RJ, Goss SP, Joseph J, Kalyanaraman B. Nitration of γ-tocopherol and oxidation of α-tocopherol by copper-zinc superoxide dismutase/H_2O_2/NO_2^-: role of nitrogen dioxide free radical. *Proc Natl Acad Sci USA* 1998; 95: 12912—12917.
18. van der Vliet A, Eiserich JP, Halliwell B, Cross CE. Formation of reactive nitrogen species during peroxidase-catalyzed oxidation of nitrite. A potential additional mechanism of nitric oxide-dependent toxicity. *J Biol Chem* 1997; 272: 7617—7625.
19. Mehl M, Daiber A, Herold S, Shoun H, Ullrich V. Peroxynitrite reaction with heme proteins. *Nitric Oxide Biol Chem* 1999; 3: 142—152.
20. Reszka KJ, Matuszak Z, Chignell CF, Dillon J. Oxidation of biological electron donors and antioxidants by a reactive lactoperoxidase metabolite from nitrite (NO_2^-): an EPR and spin trapping study. *Free Radic Biol Med* 1999; 26: 669—678.
21. Liu X, Miller MS, Joshi MS, Thomas DD, Lancaster JR Jr. Accelerated reaction of nitric oxide with O_2 within the hydrophobic interior of biological membranes. *Proc Natl Acad Sci USA* 1998; 95: 2175—2179.

22. Goss SP, Singh RJ, Hogg N, Kalyanaraman B. Reactions of NO, NO_2 and peroxynitrite in membranes: pysiological implications. *Free Radic Res* 1999; 31: 597—606.
23. Prütz WA, Mönig H, Butler J, Land EJ. Reactions of nitrogen dioxide in aqueous model systes: oxidation pf tyrosine units in peptides and proteins. *Arch Biochem Biophys* 1985; 243: 125—134.
24. Pryor WA, Lightsey JW. Mechanisms of nitrogen dioxide reactions: initiation of lipid peroxidation and the production of nitrous acid. *Science* 1981; 214: 435—437.
25. O'Donnell VB, Eiserich JP, Chumley PH, Jablonsky MJ, Krishna NR, Kirk M, Barnes S, Darley-Usmar VM, Freeman BA. Nitration of unsaturated fatty acids by nitric oxide-derived reactive nitrogen species peroxynitrite, nitrous acid, nitrogen dioxide, and nitronium ion. *Chem Res Toxicol* 1999; 12: 83—92.
26. Radi R, Beckman JS, Bush KM, Freeman BA. Peroxynitrite-induced membrane lipid peroxidation: the cytotoxic potential of superoxide and nitric oxide. *Arch Biochem Biphys* 1991; 288: 481—487.
27. Gallon AA, Pryor WA. The identification of the allylic nitrite and nitro derivatives of methyl linoleate and methyl linolenate by negative chemical ionization mass spectroscopy. *Lipids* 1993; 28: 125—133.
28. Poutet M. Procédé pour reconnaître la falsification de l'huile d'olive par celle de graines. *Annales de chimie et de physique* 1819; 12: 58—62.
29. Rubbo H, Radi R, Truhillo M, Telleri R, Kalyanaraman B, Barnes S, Kirk M, Freeman BA. Nitric oxide regulation of superoxide and peroxynitrite-dependent lipid peroxidation. Formation of novel nitrogen-containing oxidized lipid derivatives. *J Biol Chem* 1994; 269: 26066—26075.
30. O'Donnell VB, Eiserich JP, Bloodsworth A, Chumley PH, Kirk M, Barnes S, Darley-Usmar VM, Freeman BA. Nitration of unsaturated fatty acids by nitric oxide-derived reactive species. *Methods Enzymol* 1999; 301: 454—470.
31. Lai CC, Finlayson-Pitts BJ. Reactions of dinitrogen pentoxide and nitrogen dioxide with 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine. *Lipids* 1991; 26: 306—314.
32. Morrow JD, Roberts LJ. The isoprostanes: unique bioactive products of lipid peroxidation. *Prog Lipid Res* 1997; 36: 1—21.
33. Harrison KA, Murphy RC. Isoleukotrienes are biologically active free radical products of lipid peroxidation. *J Biol Chem* 1995; 270: 17273---17278.
34. McIntyre TM, Zimmerman GA, Prescott SM. Biologically active oxidized phospholipids. *J Biol Chem* 1999; 274: 25189—25192.
35. Zimmerman GA, Prescott SM, McIntyre TM. Oxidatively fragmented phospholipids as inflammatory mediators: the dark side of polyunsaturated lipids. *J Nutr* 1995; 125: 1661S—1665S.
36. Balazy M. Peroxynitrite and arachidonic acid. Identification of arachidonate epoxides. *Pol J Pharmacol* 1994; 46: 593—600.
37. Balazy M, Iesaki T, Park JL, Kaminski PM, Wolin MS. Vicinal nitrohydroxyeicosatrienoic acids- new vasodilator lipids formed from nitrogen dioxide and arachidonic acid. *Circulation* 1999; 100: I-814-Abstract # 4297.
38. Berdeaux O, Chardigny JM, Sébédio J-L, Mairot T, Poullain D, Vatelè J-M, Noel JP. Effects of a trans isomer of arachidonic acid on rat platelet aggregation and eicosanoid production. *J Lipid Res* 1996; 37: 2244—2250.
39. Portoles MT, Ainaga MJ, Pagani R. The induction of lipid peroxidation by E. coli lipopolysaccharide on rat hepatocytes as an important factor in the etiology of endotoxic liver damage. *Biochim Biophys Acta* 1993; 1158: 287—292.
40. Patrignani P, Santini G, Panara MR, *et al.* Induction of prostaglandin endoperoxide synthase-2 in human monocytes associated with cyclo-oxygenase-dependent F_2-isoprostane formation. *Br J Pharmacol* 1996; 118: 1285—1293.

41. Flesch M, Kilter H, Cremers B, Laufs U, Sudkamp M, Ortmann M, Muller FU, Bohm M. Effects of endotoxin on human myocardial contractility involvement of nitric oxide and peroxynitrite. *J Am Coll Cardiol* 1999; 33: 1062—1070.
42. Astiz ME, Rackow EC. Septic shock. *Lancet* 1998; 351: 1501—1505.
43. Salvemini D, Riley DP, Lennon PJ, Wang ZQ, Currie MG, Macarthur H, Misko TP. Protective effecs of a superoxide dismutase mimetic and peroxynitrite decomposition catalysts in endotoxin-induced intestinal damage. *Br J Pharmacol* 1999; 127: 685—692.
44. Wolkow PP, Jankowska E, Bartus JB, Uracz W, Gryglewski RJ: Endotoxin-induced acute respiratory distress syndrome in nitric oxide-deficient rats. In: Eicosanoids, Aspirin, and Asthma. Szczeklik A, Gryglewski RJ, Vane JP, eds. Marcel Dekker, 1998; 273—282.
45. Szabo C. Role of nitric oxide in endotoxic shock. An overview of recent advances. *Ann NY Acad Sci* 1998; 851: 422—425.
46. Leach M, Frank S, Olbrich A, Pfeilschifter J, Thiemermann C. Decline in the expression of copper/zinc superoxide dismutase in the kidney of rat with endotoxic shock: effects of the superoxide anion radical scavenger, tempol, on organ injury. *Br J Clin Pharmacol* 1998; 125: 817—825.
47. Brown JM, Grosso MA, Terada LS, Whitman GJ, Banerjee A, White CW, Harken AH, Repine JE. Endotoxin pretreatment increases endogenous myocardial catalase activity and decreases ischemia-reperfusion injury of isolated rat hearts. *Proc Natl Acad Sci USA* 1989; 86: 2516—2520.
48. Broner CW, Shenep JL, Stidham GL, Stokes DC, Fairclough D, Schonbaum GR, Rehg JE, Hildner WK. Effect of antioxidants in experimental Escherichia coli septicemia. *Circ Shock* 1989; 29: 77—92.
49. Peters A, Liu E, Verrier RL, *et al.* Air pollution and incidence of cardiac arrhythmia. *Epidemiology* 2000; 11: 11—17.
50. Ichinose T, Sagai M. Studies on biochemical effects of nitrogen dioxide. III. Changes of the antioxidative protective systems in rat lungs and of lipid peroxidation by chronic exposure. *Toxicol Appl Pharmacol* 1982; 66: 1—8.
51. Halliwell B, Gutteridge JMC. Free radicals in biology and medicine. Oxford University Press, 3rd Edition, 1999.
52. Hooper DC, Spitsin S, Kean RB, Champion JM, Dickson GM, Chaudhry I, Koprowski H. Uric acid, a natural scavenger of peroxynitrite, in experimental allergic encephalomyelitis and multiple sclerosis. *Proc Natl Acad Sci USA* 1998; 95: 675—680.
53. Becker BF, Reinholz N, Ozcelik T, Leipert B, Gerlach E. Uric acid as radical scavenger and antioxidant in the heart. *Pflugers Arch* 1989; 415: 127—135.
54. Xie YW, Wolin MS. Role of nitric oxide and its interaction with superoxide in the suppression of cardiac muscle mitochondrial respiration. Involvement in response to hypoxia/reoxygenation. *Circulation* 1996; 94: 2580—2586.

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Author's address: Michael Balazy, Ph. D. New York Medical College, Department of Pharmacology, Valhalla, NY 10595, USA.
E-mail: Michael_Balazy@.nymc.edu