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Original Contribution

Nitrated lipids decompose to nitric oxide and lipid radicals and cause vasorelaxation

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Abstract

Nitric oxide-derived oxidants such as nitrogen dioxide and peroxynitrite have been receiving increasing attention as mediators of nitric oxide toxicity. Indeed, nitrated and nitrosated compounds have been detected in biological fluids and tissues of healthy subjects and in higher yields in patients under inflammatory or infectious conditions as a consequence of nitric oxide overproduction. Among them, nitrated lipids have been detected in vivo. Here, we confirmed and extended previous studies by demonstrating that nitrolinoleate, chlolesteryl nitrolinoleate, and nitrohydroxylinoleate induce vasorelaxation in a concentration-dependent manner while releasing nitric oxide that was characterized by chemiluminescence- and EPR-based methodologies. As we first show here, diffusible nitric oxide production is likely to occur by isomerization of the nitrated lipids to the corresponding nitrite derivatives that decay through homolysis and/or metal ion/ascorbate-assisted reduction. The homolytic mechanism was supported by EPR spin-trapping studies with 3,5-dibromo-4-nitrosobenzenesulfonic acid that trapped a lipid-derived radical during nitrolinoleate decomposition. In addition to provide a mechanism to explain nitric oxide production from nitrated lipids, the results support their role as endogenous sources of nitric oxide that may play a role in endothelium-independent vasorelaxation.

Keywords: Lipid peroxidation; Nitrogen dioxide; EPR; Nitrolinoleate; Cholesteryl nitrolinoleate; Vasodilation

Introduction

Nitrated and nitrosated compounds have been shown to be produced when proteins, thiols, and lipids are exposed to $^{\bullet}NO/^{\bullet}NO_2$ and peroxynitrite, both in vitro and in vivo [1–3]. Such compounds are potentially involved with $^{\bullet}NO$ toxicity in a variety of pathophysiological conditions [4]. Indeed, nitration of proteins, DNA, and low molecular weight compounds has been shown to be a consequence of oxidative damage associated with nitrosoative stress, a recurrent event associated with inflammatory and infectious conditions [5].

Oxidized and nitrated lipids that are likely to be produced in vivo have been shown to be generated during LDL oxidation by transition metal ions, exposure of lipids to $^{\bullet}NO_2$ or peroxynitrite in vitro and in cell cultures [6–10]. Also relevant, the fast diffusion-controlled reaction between lipid radicals and $^{\bullet}NO$ may ultimately lead to the production of a variety of nitrated lipid derivatives [11–13]. This

Abbreviations: carboxi-PTIO-2-(4-carboxyphenyl)-4,4,5,5-tetramethylimidazoline-1-oxyl 3 oxide; $ChLNO_2$, cholesteryl nitrolinoleate; DBNBS, 3,5-dibromo-4-nitrosobenzenesulfonic acid; EPR, electronic paramagnetic resonance; Hb, rabbit hemoglobin; MGD, *N*-methylglucamine dithiocarbamate; SDS, sodium dodecyl sulfate; HPLC, high-pressure liquid chromatography; LA, linoleate; LC-ESI/MS/MS, liquid chromatography/electrospray ionization tandem mass spectrometry; LDL, low-density lipoprotein; LNO_2, nitrolinoleate; LONO, nitritelinoleate; LNO_2OH, nitrohydroxylinoleate; NMR, nuclear magnetic resonance spectroscopy.

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reaction participates in the lipid peroxidation chain-breaking mechanism accounting for the suggested •NO antioxidant property [14–16].

Actually, several nitrated compounds have been demonstrated to exert diverse biological activities by mechanisms that remain debatable. Nitrolinoleate (LNO_2) displays cell-signaling activities that appear to be anti-inflammatory leading to inhibition of platelet function and neutrophil superoxide generation through cAMP-dependent mechanisms [17,18]. Indeed, LNO_2 [17,18] and nitrohydroxyarachidonate [19] have been shown to exhibit vasorelaxatory effects in vitro, an observation consistent with •NO production. Moreover, LNO_2, nitrohydroxylinoleate ($LONO_2$) [20] and cholesteryl nitrolinoleate ($ChLNO_2$) [21] have been detected in the human blood plasma and lipoproteins of normolipidemic and hyperlipidemic subjects evidencing their formation in vivo.

In this context, this study using mass spectrometry and chemiluminescence- and EPR-based methodologies clearly shows that LNO_2, LNO_2OH, and $ChLNO_2$ spontaneously decay, producing •NO and probably carbon-centered radicals at room temperature. Here, we demonstrate diffusible •NO production from nitrated lipids based on several lines of evidence. First, it was possible to confirm that LNO_2 induces vasorelaxation. In addition, a soluble guanilyl cyclase inhibitor, ODQ, efficiently inhibited LNO_2-induced vasorelaxation. Second, time-dependent nitric oxide release was observed by chemiluminescence measurements and ascorbate-stimulated chemiluminescence was shown to correlate with LNO_2 consumption followed by mass spectrometry unambiguously characterizing LNO_2 as source of •NO. Third, we provided spectroscopic evidence for •NO release from nitrated lipids by incubations of nitrated lipids with $MGD_2Fe(II)$ and 2-phenyl-4,4,5,5,-tetramethylimidazoline-1-oxyl-3-oxide (carboxy-PTIO) provided expected EPR-active products of •NO. Also, a hypothesis about the mechanisms of decomposition and •NO release by nitrated lipids is discussed.

Experimental procedures

Materials

2-Phenyl-4,4,5,5,-tetramethylimidazoline-1-oxyl-3-oxide was purchase from Calbiochem (San Diego, CA). Sodium [^{15}N] nitrite and nitronium tetrafluoroborate were purchased from Aldrich Chemical Co (Milwaukee, WI). 2-Propanol and chromatographic grade methanol were obtained from Merck (Darmstadt, Germany). All other reagents were from Sigma Chemical Co (St. Louis, MO). DBNBS was synthesized from 3,5 dibromosulfanilic acid purchased from Aldrich Chemical Co. (Milwaukee, WI) as previously described [22]. Sodium *N*-methyl-D-glucamine dithiocarbamate (MGD) and iron (II) sulfate heptahydrate were obtained from OMRF spin-trap source (Oklahoma City, OK) and were mixed in a 2:1 ratio to prepare the iron complex just before each set of experiments.

Synthesis and characterization of nitrated lipid derivatives

LNO_2 and $ChLNO_2$ were obtained from their precursors linoleate and cholesteryl linoleate, respectively, as previously described [20,21]. Briefly, the lipids were reacted with NO_2BF_4 under agitation and reduced oxygen tensions. Then, the products were purified, by passing the mixture through a silica cromatographic column. The fractions were analyzed by mass spectrometry and those presenting the highest concentrations of purified LNO_2 or $ChLNO_2$ were mixed and evaporated under vacuum before freezing at −80°C. LNO_2OH was prepared according to Lima et al. [20]. Nitration of the hydroperoxylinoleate was achieved by incubating the lipid with acidified nitrite solutions for 15 min under air. The products were purified by liquid cromatography and characterized by mass spectrometry as previously described. The fractions containing the highest yield of purified LNO_2OH were mixed and evaporated under vacuum. The obtained product was kept frozen at −80°C.

Vasorelaxation of rat aortic rings

Thoracic aortas from male Wistar rats were carefully removed and freed from all periadventitial tissue. Aortic rings (ca. 5 mm) were mounted in organ chambers in Krebs-Henseleit solution (in mmol/L: $CaCl_2$ 1.6, $MgSO_4$ 1.17, EDTA 0.026, NaCl 130, $NaHCO_3$ 14.9, KCl 4.7, KH_2PO_4 1.18, glucose 11), at 37°C and were connected to force transducers (Biopac System TSD 105^A, USA). The solution was bubbled with a carbogenic mixture (95% O_2/5% CO_2). The resting tension was set at 1.5 g and after a 60-min equilibration period. Vessels were precontracted with noradrenaline 1×10^{-7} M and thereafter exposed to different amounts of nitrated lipids synthesized. Control experiments were performed in which ODQ (a guanylate cyclase inhibitor, at 100 μM concentration) was added after noradrenaline. For experiments with endothelium-denuded arteries, preparations were rubbed in their internal surface with a cotton-wrapped stick. Cumulative concentration-effect curves with each nitrated lipid were made for intact or denuded aortic rings. Control experiments were performed with the nonnitrated lipids at comparable concentrations.

Determination of •NO release by chemiluminescense

The •NO release was measured employing a •NO chemiluminescence analyzer (model NOA, Sievers Instruments, Boulder, CO). It was studied in the absence and presence of the •NO-trapping agents carboxy-PTIO and rabbit hemoglobin, and the presence of the reducing agent ascorbate. Briefly, LNO_2 or LNO_2OH was injected into the chamber of the home-built apparatus which contained a 2-ml final volume of phosphate buffer (25 mM, pH 7.4, 0.5% of