Available online at www.sciencedirect.com

SCIENCE DIRECT

Free Radical Biology & Medicine 39 (2005) 1162 – 1176

www.elsevier.com/locate/freeradbiomed

Original Contribution

Induction of endothelial cell apoptosis by lipid hydroperoxide-derived bifunctional electrophiles

Wenying Jian[a], Jasbir S. Arora[a], Tomoyuki Oe[a], Vladimir V. Shuvaev[b], Ian A. Blair[a,*]

[a]*Center for Cancer Pharmacology, University of Pennsylvania School of Medicine, Philadelphia, PA 19104-6160, USA*
[b]*Institute for Environmental Medicine, University of Pennsylvania School of Medicine, Philadelphia, PA 19104-6160, USA*

Received 11 October 2004; revised 13 June 2005; accepted 13 June 2005

Abstract

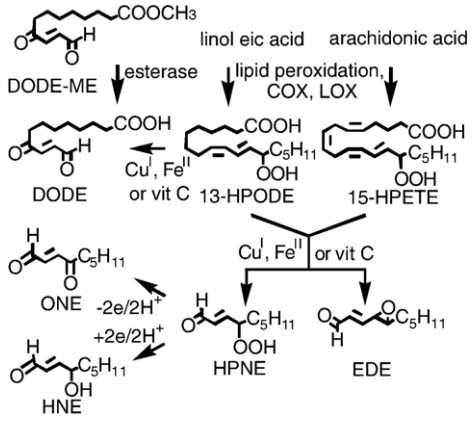
Endothelial dysfunction is considered to be the earliest event in atherogenesis. Oxidative stress, inflammation, and apoptosis play critical roles in its progression and onset. Lipid peroxidation, which occurs during oxidative stress, results in the formation of lipid hydroperoxide-derived bifunctional electrophiles such as 4-hydroxy-2(*E*)-nonenal that induce apoptosis. In this study, recently identified lipid hydroperoxide-derived bifunctional electrophiles 4-oxo-2(*E*)-nonenal (ONE; 5–30 μM) and 4,5-epoxy-2(*E*)-decenal (EDE; 10–20 μM) were shown to cause a dose- and time-dependent apoptosis in EA.hy 926 endothelial cells. This was manifest by morphological changes, caspase-3 activation, and poly(ADP-ribose) polymerase cleavage. Bifunctional electrophiles caused cytochrome *c* release from mitochondria into the cytosol, implicating a mitochondrial pathway of apoptosis in the endothelial cells. The novel carboxylate-containing lipid hydroperoxide-derived bifunctional electrophile 9,12-dioxo-10(*E*)-dodecenoic acid was inactive because it could not translocate across the plasma membrane. However, its less polar methyl ester derivative (2–10 μM) was the most potent inducer of apoptosis of any bifunctional electrophile that has been tested. An acute decrease in intracellular glutathione (GSH) preceded the onset of apoptosis in bifunctional electrophile-treated cells. The ability of ONE and EDE to deplete GSH was directly correlated with their predicted reactivity toward nucleophilic amino acids. Liquid chromatography/mass spectrometry methodology was developed in order to examine the intracellular and extracellular concentrations of bifunctional electrophile-derived GSH adducts. Relative intracellular/extracellular ratios of the GSH adducts were identical with the rank order of potency for inducing caspase 3 activation. This suggests that there may be a role for the bifunctional electrophile-derived GSH adducts in the apoptotic response. *N*-Acetylcysteine rescued bifunctional electrophile-treated cells from apoptosis, whereas the GSH biosynthesis inhibitor D,L-buthionine-(*R*,*S*)-sulfoximine sensitized the cells to apoptosis. These data suggest that lipid hydroperoxide-derived bifunctional electrophiles may play an important role in cardiovascular pathology through their ability to induce endothelial cell apoptosis.

Keywords: Lipid peroxidation; Bifunctional electrophiles; Apoptosis; Glutathione; Endothelial cells; Free radicals

Polyunsaturated fatty acids (PUFAs) can be converted into lipid hydroperoxides either enzymatically by the action of cyclooxygenases (COXs) [1] or lipoxygenases (LOXs) [2] or nonenzymatically by the action of reactive oxygen species (ROS) [3]. Thus, linoleic acid is oxidized to 13-hydroperoxy-9,11-(*Z*,*E*)-octadecadienoic acid (13-HPODE; Scheme 1), whereas arachidonic acid is oxidized to 15-hydroperoxy-5,8,11,13-(*Z*,*Z*,*Z*,*E*)-eicosatetraenoic acid (15-HPETE; Scheme 1). These ω-6 PUFA lipid hydroperoxides can then undergo Fe(II), Cu(I), or vitamin C-mediated homolytic decomposition to form α,β-unsaturated aldehydes through three distinct pathways (Scheme 1) [4–6]. The first pathway, which most likely involves α-cleavage of an alkoxy radical, results in formation of 4,5-epoxy-2(*E*)-decenal (EDE) [6–8]. The second pathway involves formation of 4-hydroperoxy-2(*E*)-nonenal (HPNE), which undergoes dehydration to 4-oxo-2(*E*)-nonenal (ONE) or reduction into 4-hydroxy-2(*E*)-nonenal (HNE) [6,9,10]. The third pathway, which we have recently identified [11],

* Corresponding author. Fax: +1 215 573 9889.
E-mail address: ian@spirit.gcrc.upenn.edu (I.A. Blair).

0891-5849/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.freeradbiomed.2005.06.008



Scheme 1. Lipid hydroperoxide-mediated formation of the α,β-unsaturated aldehyde bifunctional electrophiles EDE, HPNE, HNE, ONE, and DODE.

involves the formation of 9,12-dioxo-10(*E*)-decenoic acid (DODE). This pathway occurs exclusively with 13-HPODE, the ω-6 lipid hydroperoxide derived from linoleic acid (Scheme 1). DODE has also been detected as a breakdown product of 9-hydroperoxy-10,12-octadecadienoic acid from lentil seeds [12]. It is a more polar bifunctional electrophile than EDE, HPNE, HNE, or ONE because it contains a terminal carboxylate moiety.

Lipid hydroperoxide-derived bifunctional electrophiles can covalently bind to macromolecules such as proteins and DNA, which can disrupt cellular homeostasis and cause cellular damage. The most intensively studied lipid hydroperoxide-derived bifunctional electrophile is HNE [13,14]. It causes apoptosis in diverse cell types such as colorectal carcinoma cells [15], neuronal cells [16], macrophages [17], erythroleukemia cells [18], vascular smooth muscle cells [19], and endothelial cells [20]. ONE was recently identified as a major homolytic decomposition product of lipid hydroperoxides [9]. It has a structure similar to that of HNE except that it contains a C-4-oxo group instead of a C-4-hydroxyl group (Scheme 1). EDE contains a 4,5-epoxy group instead of the C-4-hydroxyl group in HNE (Scheme 1). DODE has the same α,β-unsaturated aldehyde that is present in ONE and EDE but has a slightly longer methylene chain that terminates with a carboxyl group (Scheme 1). A previous study of structure–activity relationships in HNE-induced apoptosis in a macrophage cell line revealed that the α,β-unsaturated aldehyde was critical for activity [21]. Another factor affecting the cytotoxicity was the length of the alkyl chain attached to the α,β-unsaturated aldehyde. Increasing the chain length caused increased toxicity, presumably because of increased transport across the plasma membrane [21]. ONE, EDE, and DODE all contain the α,β-unsaturated aldehyde. This suggested that they would be capable of inducing apoptosis in endothelial cells. Furthermore, a recent study has demonstrated that ONE can induce apoptosis in colorectal cancer cells with a potency similar to that of HNE [22].

Classic risk factors for atherosclerosis include oxidized low-density lipoprotein (oxLDL) [23] and oxidative stress [24,25]. The oxLDL contains lipid hydroperoxide-derived bifunctional electrophiles such as HNE that are bound to protein [26]. Oxidation-specific epitopes are generated on both oxLDL and apoptotic cells [23]. Many of these same epitopes serve as ligands that mediate the binding and clearance of oxidatively damaged lipoprotein particles and apoptotic cells. In addition, other epitopes of oxidized LDL play a role in immune activation, an important mechanism in atherosclerosis [23]. There are increased levels of LOX and COX in atherosclerotic plaques [27–29]. Furthermore, enhanced formation of lipid peroxidation products such as isoprostanes and HNE has been observed in the plaques [30,31]. These studies provide compelling evidence of a link between lipid peroxidation and atherosclerosis. Apoptosis has been observed in different cell types involved in atherosclerosis, including macrophages, vascular smooth muscle cells, and endothelial cells [32]. Endothelial injury or dysfunction is one of the first events to occur in atherogenesis. HNE causes endothelial apoptosis and dysfunction, including abnormal cytokine production, diminished cell viability, and impaired endothelial barrier function [20,33]. HNE-mediated apoptosis in colorectal and lymphoma cells lines seems to be mediated by decreased intracellular glutathione (GSH) concentrations [15,34–36]. The aim of the present study was to determine whether other recently identified lipid hydroperoxide-derived bifunctional electrophiles [8,9,11] are equally as potent as HNE and to examine the role that GSH plays in the apoptotic process.

Materials and methods

Materials

All chemicals, *N*-acetylcysteine (NAC), D,L-buthionine-(*R*,*S*)-sulfoximine (BSO), trifluoroacetic acid (TFA), the cocktail of protease inhibitors for mammalian tissue, and horseradish peroxidase-conjugated goat anti-rabbit antibody were obtained from Sigma (St. Louis, MO, USA). The EA.hy 926 endothelial cells were a generous gift from Dr. Cora Edgell (University of North Carolina). Dulbecco's minimal essential medium (DMEM) was from Gibco (Grand Island, NY, USA), and fetal bovine serum (FBS) was from U.S. Biotechnologies (Parker Ford, PA, USA). BCA protein assay reagent was obtained from Pierce Biotechnology (Rockford, IL, USA). Precast 12% NuPAGE Bis–Tris gels, precast 7% NuPAGE Novex Tris–acetate gels, and 0.45-μm nitrocellulose membranes were obtained from Invitrogen (Carlsbad, CA, USA). Anti-human caspase-3 (clone 31A1067) was from Imgenex (San Diego, CA, USA). Rabbit anti-human poly(ADP-ribose) polymerase (PARP) was from Roche Molecular Biochemicals (Germany). Rabbit anti-human cytochrome *c* antibody and peroxidase-conjugated goat anti-mouse antibody were from Bio-Rad (Hercules, CA, USA). The enhanced chemiluminescence (ECL) Western blotting reagent was supplied by Amersham (Arlington