Fragmented oxidation products define barrier disruptive endothelial cell response to OxPAPC

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Excessive concentrations of oxidized phospholipids (OxPL), the products of 1-palmitoyl-2-arachidonoyl-*sn*-glycero-3-phosphatidylcholine (PAPC) oxidation have been detected in atherosclerosis, septic inflammation, and acute lung injury (ALI) and have been shown to induce vascular barrier dysfunction. In contrast, oxidized PAPC (OxPAPC) at low concentrations exhibit potent barrier protective effects. The nature of such biphasic effects remains unclear. We tested the hypothesis that barrier-disruptive effects of high OxPAPC doses on endothelial cell (EC) monolayer are defined by fragmented products of PAPC oxidation (lysophosphatidyl choline (lyso-PC), 1-palmitoyl-2-(5-oxovaleroyl)-*sn*-glycero-phosphatidylcholine (POVPC), 1-palmitoyl-2-glutaroyl-*sn*-glycero-phosphatidylcholine (PGPC)), whereas barrier enhancing effects are mediated by full length oxidated PAPC products and may be reproduced by single compounds contained in the OxPAPC such as 1-palmitoyl-2-(5,6-epoxyisoprostane E2)-sn-glycero-3-phosphatidyl choline (PEIPC). All 3 fragmented OxPAPC products increased EC permeability in a dose-dependent manner, whereas PEIPC decreased it and reversed barrier disruptive effects of lyso-PC, POVPC, and PGPC monitored by measurements of transendothelial electrical resistance. Immunofluorescence staining and western blot analysis showed that PGPC mimicked the cytoskeletal remodeling and tyrosine phosphorylation of adherens junction (AJ) protein vascular endothelial (VE)-cadherin leading to EC barrier dysfunction induced by high OxPAPC concentrations. Barrier-disruptive effects of PGPC were abrogated by reactive oxygen species (ROS) inhibitor, N-acetyl cysteine, or Src kinase inhibitor, PP-2. The results of this study show that barrier disruptive effects of fragmented OxPAPC constituents (lyso-PC, POVPC, PGPC) are balanced by barrier enhancing effects of full length oxygenated products (PEIPC). These data strongly suggest that barrier disruptive effects of OxPAPC at higher concentrations are dictated by predominant effects of fragmented phospholipids such as PGPC, which promote ROS-dependent activation of Src kinase and VE-cadherin phosphorylation at Tyr^{658} and Tyr^{731} leading to disruption of endothelial cell AJs. (Translational Research 2013;161:495–504)

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Supported by HL087823, HL076259 (K.G.B.); HL107920 (A.A.B.) and HL064731 (L.K., J.A.B.).

Conflict of interest: The authors have no conflicts of interest to declare. All authors have read the journal's policy on disclosure of potential conflicts of interest.

Submitted for publication September 6, 2012; revision submitted December 2, 2012; accepted for publication December 13, 2012.

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1931-5244/$ - see front matter

http://dx.doi.org/10.1016/j.trsl.2012.12.008

Abbreviations: AJ = adherens junctions; ALI = acute lung injury; ARDS = acute respiratory distress syndrome; EC = endothelial cells; EGM = endothelial growth medium; 4-HNE = 4 –hydroxy nonenale; HPAEC = human pulmonary artery endothelial cells; HRP = horseradish peroxidase; Lyso-PC = lysophosphatidyl choline; MDA = malone dialdehyde; MLC = myosin light chains; MS = mass spectrometry; MYPT = myosine phosphatase; OxPAPC = oxidized 1-palmitoyl-2-arachidonoyl-sn-glycero-3-phosphatodyl choline; OxPL = oxidized phospholipids; PBS = phosphate buffered saline; PEIPC = 1-palmitoyl-2-(5,6-epoxyisoprostane E2)-sn-glycero-3-phosphatidyl choline; PGPC = 1-palmitoyl-2-glutaroyl-sn-glycero-phosphatidyl choline; POVPC = 1-palmitoyl-2-(5-oxovaleroyl)-sn-glycero-phosphatidyl choline; PVDF = polyvinylidene fluoride; ROS = reactive oxygen species; RT = room temperature; SDS = sodium dodecyl sulfate; TER = transendothelial electrical resistance; VE-cadherin = vascular endothelial cadherin; VEGFR2 = vascular endothelial growth factor receptor-2

AT A GLANCE COMMENTARY

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Background

Oxidative stress and activation of phospholipases lead to the formation and accumulation of biologically active lipid oxidation products. Increased levels of oxidized phospholipids present in the injured lung may influence pulmonary endothelial cell functions including modulation of pulmonary inflammatory response and endothelial barrier regulation.

Translational Significance

This study investigates differential effects of full length and fragmented products of phospholipid oxidation on vascular endothelial permeability and describes a novel mechanism of endothelial barrier compromise by fragmented compound 1-palmitoyl-2-glutaroyl-*sn*-glycero-phosphatidyl-choline (PGPC) via activation of ROS-Src-VE-cadherin pathway. These findings extend our understanding of complex role of oxidized phospholipids in the control of vascular barrier.

Elevated circulating levels of oxidized phospholipids (OxPL) have been found in a variety of pathologic conditions accompanied by oxidative stress including atherosclerosis, autoimmune diseases, lung injury and sepsis.[1] Lipid oxidation in acute respiratory distress syndrome (ARDS) is manifested by elevated concentrations of lipid peroxidation products, such as 4-hydroxy-2-nonenal and 8-isoprostane.[2] Exhaled 8-isoprostane has been even used as biomarker and diagnostic tool for evaluation of oxidative stress in patients with chronic obstructive pulmonary disease, lung injury and ARDS.[3,4] Generation of OxPL by oxidation of arachidonic acid-containing membrane phospholipids occurs upon inflammatory activation of various cell types including myeloid cells (neutrophils and monocytes), epithelium, fibroblasts, and vascular endothelium. Inflammatory events lead to formation of hydroperoxides and accumulation of fragmented and full length products of cell membrane phospholipid oxidation such as 1-palmitoyl-2-(5-oxovaleroyl)-*sn*-glycero-phosphatidyl-choline (POVPC), 1-palmitoyl-2-glutaroyl-*sn*-glycero-phosphatidylcholine (PGPC), lysophosphatidyl choline (lyso-PC), 1-palmitoyl-2-(5,6-epoxyisoprostane E2)-*sn*-glycero-3-phsphocholine (PEIPC), and others.[5,6]

Increased levels of OxPL present in the injured lung significantly influence pulmonary endothelial cell (EC) functions including inflammatory responses and barrier regulation.[7-10] However, *in vitro* and *in vivo* studies demonstrate opposite effects of high and low OxPL doses on vascular permeability and lung injury, which remain an intriguing question in vascular biology, and further understanding of this phenomenon is of great importance.

Previous reports demonstrate that 1-palmitoyl-2-arachidonoyl-*sn*-glycero-3-phosphatidyl choline (PAPC) oxidized *in vitro* induced a pronounced barrier enhancing response in vascular endothelium when present in low concentrations (5–20 μg/mL) in either pulmonary or systemic circulation.[11] In addition, these OxPAPC doses attenuated agonist-induced EC permeability and prevented pulmonary vascular leak caused by mechanical ventilation at high tidal volume.[9,11,12] These effects were due to OxPAPC-induced stimulation of small GTPases Rac1 and Rap1, which promoted enhancement of peripheral actin cytoskeleton and EC junctions.[11,13] In addition, low OxPAPC doses stimulated membrane translocation of negative regulator of Rho signaling, p190RhoGAP, which suppressed Rho pathway of vascular permeability.[14] In turn, higher OxPAPC doses exhibit opposite effects and cause rapid endothelial monolayer barrier disruption.[11,12] The nature of contrasting biologic activities of high and low OxPAPC concentrations and dose-