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EPISODIC INFLAMMATION COMPROMISES VASCULAR STRUCTURE AND FUNCTION VIA REDOX-DEPENDENT PATHWAYS

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Whereas inflammation is positively correlated with vascular disease, the mechanisms underlying this paradigm remain to be fully elucidated. To ascertain whether repetitive acute inflammatory responses contribute to progressive vascular injury, rats were injected once weekly over the course of 4 weeks with lipopolysaccharide (LPS). Following each inflammatory challenge, plasma levels of the acute-phase protein a2-macroglobulin increased dramatically over the first 48 hrs and subsided to basal levels after 7 days. This trend was apparent with each subsequent injection of LPS. While vascular function, assessed by acetylcholine-induced relaxation and phenylephrine-induced constriction of aortic tissues was not altered after a single LPS injection followed by 7 days recovery, relaxation and constrictor responses were significantly altered after 4 weekly LPS injections. Dysfunctional vascular responses were associated with increased aortic tissue levels of myeloperoxidase and protein-associated dityrosine, patterns consistent with smooth muscle cell hypertrophy and remodeling, and increased deposition of extracellular matrix and was paralleled by a ~3-fold increase in plasma angiotensin II. Whereas robust systemic nitric oxide (NO) production was observed 24 h after the first LPS injection, this response was dramatically diminished following each subsequent weekly injection. These results reveal that episodic bouts of acute inflammation, despite resolution of each intermittent inflammatory response, lead to progressive impairment of vascular structure and function that appears to be mediated by alterations in interrelated redox-dependent pathways.

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EFFECTS OF EXOGENOUS SUPPLY OF REDUCING AGENTS ON RHINOVIRUS HRV16 ACTIVITY

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Infections caused by rhinovirus (Picornaviridae) often superimpose to pathologies such as asthma and chronic obstructive pulmonary disease (COPD). The rhinovirus HRV16 uses as a receptor the glycoprotein ICAM-1, which plays a relevant role in activation of leukocytes during inflammatory processes and oxidative stress. Flogosis due to HRV16 increases expression of ICAM-1, causing a further local diffusion of rhinovirus. The effects of exogenous supply of reducing agents (sulphur-rich thermal water or reduced glutathione, GSH) were investigated in cultured human lung pneumocytes (line A549) exposed to HRV16. Cell homogenates were examined by spectrophotometric analysis of cytochrome c reduction in presence of superoxide dismutase and a serine protase inhibitor, phenylmethyl sulphonyl fluoride. Exposure to HRV16 caused an additional superoxide production in cultured lung cells, quenched by preincubation with thermal water or GSH. Analysis of cell homogenates by reversed phase high performance liquid chromatography (RP-HPLC) confirmed that an exogenous supply of reducing agents could significantly increase intracellular concentration of GSH, thus lowering oxygen radical production caused by exposure of cells to HRV16.

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INFLAMMATORY RESPONSE AND REDOX ALTERATIONS IN A RAT MODEL OF LUNG HEMORRHAGIC INJURY

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Increase in redox activity and activation of iron turnover (IrT) are essential patterns of a cascade of events leading to resolution of traumatic hemorrhagic inflammatory lesions (HIL). The objectives of the present study were spatio-temporal assessment of transmigration of inflammatory leukocytes (LKC), deposition of hemeoxygenase-1 (HO-1), myeloperoxidase (MPO), Cu,Zn-SOD (SOD-1), transferrin (TRF), and 3-nitrotyrosine (3NTyr), in conjunction with dynamics of redox IrT in a rat model of lung trauma (LT). LHI was induced by exposure of animals to shock wave (SW). Sections of HIL were assessed with histology, electron microscopy, EPR spectroscopy, immunofluorescence imaging, and immunoblotting. Accumulation of HO-1, MPO, 3NTyr, SOD-1 in HIL at the first 12 h was due to transmigration of LKC into injured areas. At that time, formation of 3NTyr in alveolar endothelium correlated with rearrangement of VE-CDH. Expression of HO-1 and SOD-1 was observed in endothelial and alveolar epithelial cells at 24 h post injury. Deposition and biodegradation of extravasated hemoglobin (exvHb) in alveolar space, LKC, and lung cells occurred after 12 h postexposure. These alterations were accompanied by time-dependent conversion of EPR signals of heme-iron d5Fe^{3+} and d3Fe^{3+} complexes to the signals of non-heme iron d5Fe^{3+} and d3Fe^{3+} complexes with substantial increase in EPR signal of TRF-bound [Fe^{3+}]. Transmigration of LKC and formation of reactive nitrogen and oxygen species and iron deposition in endothelial and epithelial cells were accompanied by destruction of alveolar capillary network and necrosis of the pulmonary epithelial cells following 24 h postexposure.

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IRON-RICH SINGLE WALLED CARBON NANOTUBES ARE EFFECTIVE CATALYSTS OF OXIDATIVE STRESS IN RAW 264.7 MACROPHAGE CELL CULTURE MODEL: INTERACTIONS WITH INFLAMMATORY RESPONSE AND IN VIVO IMPLICATIONS.

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Single walled carbon nanotubes (SWCNT) are new emerging materials. Their manufacturing includes catalysis on iron particles. SWCNT induced inflammation provides a redox milieu in which iron can synergistically enhance damage to cells/tissue. RAW 264.7 macrophages were used to characterize the cell ability to respond to iron-rich/deplete SWCNT. EPR spectroscopy found that iron-rich (but not iron-deplete) SWCNT displayed a broad signal with g value 2.0 and half-width of 640G attributable to high spin Fe^{+3}. Co-incubation of macrophages with SWCNT resulted in their engulfment detectable by TEM. Depletion of GSH and slightly increased number of apoptotic cells in response to SWCNT was observed. No intracellular production of superoxide or NO was triggered by SWCNT as evidenced by flow cytometry with DHE and DAF-2-DA, respectively. EPR spin-trapping demonstrated that iron-rich (but not iron-deplete) SWCNT were redox-active and converted superoxide radicals produced by zymosan-stimulated marophages into hydroxyl radicals. Similarly, superoxide extracellularly generated by xanthine oxidase/xanthine yielded hydroxyl radicals. Iron-rich SWCNT oxidized ascorbate to its radical. These in vitro results are in line with marked

inflammatory response and early onset of fibrotic changes in the lungs of C57BL/6 mice exposed to SWCNT.

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MYELOPEROXIDASE-DEPENDENT MODULATION OF THE OXYLIPIN METABOLOME DURING ACUTE INFLAMMATION

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Myeloperoxidase (MPO) is an abundant phagocyte-derived hemoprotein that contributes to oxidative injury during inflammation and has been demonstrated to serve as a powerful predictor of vascular disease risk. Herein we utilized a metabolomic approach employing liquid chromatography-mass spectrometry (LC-MS) to characterize a comprehensive array of enzymatic- and free radical-derived linoleic and arachidonic acid oxidation products in wild-type and MPO-deficient mice exposed to lipopolysaccharide. We report that MPO plays a key role in modulating the spectrum of fatty acid oxylipid metabolites during acute inflammation in mice. Among the 65 fatty acid 'oxylipins' assessed in blood plasma 24 hrs after LPS injection, significantly decreased levels of the epoxides of linoleic acid (leukotoxin) were detected in MPO-deficient mice compared to wild-type mice. Consistent with this notion, fatty acid diols of both linoleic and arachidonic acid were decreased accordingly. These data suggest that MPO can directly function as a fatty acid epoxygenase, and this may be responsible for leukotoxin synthesis by neutrophils. In contrast, significantly higher levels of leukotrienes (mainly LTB4) with well-known pro-inflammatory properties were observed in MPO-deficient mice, a result likely due to the oxidative destruction of leukotrienes by MPO. Our results reveal that MPO, either directly or indirectly, modulates the balance of pro- and anti-inflammatory lipid mediators during acute inflammation.

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GLYCOSAMINOGLYCAN-DEPENDENT SEQUESTRATION OF MYELOPEROXIDASE WITHIN EXTRACELLULAR MATRIX

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Myeloperoxidase (MPO) is a highly cationic hemoprotein abundantly expressed by phagocytes that plays important roles in tissue injury associated with acute and chronic inflammation. Following secretion from activated leukocytes, MPO binds to endothelial cells and undergoes transcytotic migration to the underlying extracellular matrix (ECM) where it catalyzes various oxidative reactions and compromises vascular nitric oxide (NO) signaling. However, the molecular mechanisms governing the binding of MPO to ECM proteins, and the effect this has on its enzymatic functions remains poorly characterized. Herein, we demonstrate that MPO avidly binds to cell-derived and purified ECM proteins by a mechanism dependent upon glycosaminoglycans, and this enhances the enzymatic activity of MPO. ECM derived from various cell types facilitated binding of MPO, which was enhanced by preincubation of isolated ECM proteins with heparin, but not chondroitin sulfate. In contrast, excess heparin or chondroitin sulfate present in the exposure medium inhibits binding of MPO to cell-derived ECM proteins. These observations were confirmed with the exemplary ECM proteins fibronectin and type IV collagen. Studies utilizing ECM derived from wild-type and mutant Chinese hamster ovary cells revealed that MPO preferentially binds to heparan- and chondroitin-proteoglycans. When incubated with fibronectin or collagen, the oxidizing potential of MPO is enhanced, but its capacity to consume NO remained unchanged. Collectively, the data presented herein reveal that MPO is sequestered within ECM proteins in glycosaminoglycan-dependent manner, and that the enzymatic activity of MPO is increased when associated with ECM proteins. These results provide novel insights into the biological compartmentalization and activity of MPO during acute and chronic inflammation.

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MYELOPEROXIDASE (MPO) SERVES AS AN AUTOCRINE AND PARACRINE MEDIATOR OF NEUTROPHIL ACTIVATION BY ASSOCIATION WITH CD 11B/CD18 INTEGRINS

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Recruitment and activation of polymorphonuclear neutrophils (PMN) reflects a primary immunological response to invading pathogens and has also emerged as a hallmark of vascular inflammation. One of the principal enzymes released upon PMN activation is myeloperoxidase (MPO), a heme protein, which not only generates cytotoxic oxidants but also impacts deleteriously on nitric oxide (NO)-dependent signaling cascades within the vasculature. Since MPO also associates with the membrane of PMN, we evaluated whether MPO could also function as a proinflammatory autocrine modulator of PMN activation. The extent of membrane-associated MPO on PMN was elevated in patients with acute inflammatory vascular disease as compared to non-diseased individuals. Isolated PMN bound free MPO by a mechanism dependent upon the CD11b/CD18 integrin. PMN exposed to MPO were characterized by increased tyrosine phosphorylation, p38 MAP kinase activation, and nuclear translocation of NFkB. Binding of PMN to MPO-coated fibronectin-surfaces amplified PMN degranulation. Myeloperoxidase also augmented PMN-dependent superoxide ($O_2^{\bullet-}$) production, which was further elevated in PMN from patients with hereditary MPO deficiency. Collectively, our results reveal that binding of MPO to CD11b/CD18 integrins stimulates intracellular signaling pathways in PMN and ultimately induces PMN activation, which is independent of MPO catalytic activity. These cytokine-like properties of MPO may represent an additional explanation for its proinflammatory characteristics in vascular disease.

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EFFECT OF QUERCETIN AND 3'-*O*-METHYLQUERCETIN ON ADHESION MOLECULE AND MCP-1 EXPRESSION IN HUMAN AORTIC ENDOTHELIAL CELLS

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Dietary intake of flavonoids appears to be inversely correlated with the risk of coronary heart disease. We studied the effects of quercetin (Q) and its metabolite 3'-*O*-methylquercetin (MeQ) on TNF-α-mediated inflammatory responses in human aortic endothelial cells (HAEC). HAEC were incubated with flavonoids (17.5 h) and subsequently co-treated with 100 U/ml TNF-α (7.5 h). Q and MeQ (5-15 μM) significantly and dose-dependently attenuated cell surface expression of the adhesion molecules, E-selectin and ICAM-1, but not VCAM-1. Q and MeQ also significantly inhibited the expression of monocyte chemoattractant protein-1. To explore potential underlying mechanism(s), we used pharmacological inhibitors of the cellular signaling pathways

INFLAMMATION