Studies into the molecular mechanisms of oxidative stress-induced endothelial dysfunction and its prevention

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Background: Endothelial dysfunction is a clinically relevant feature of cardiovascular disease that is manifested as the impaired bioactivity of nitric oxide (NO) produced by the endothelial isoform of nitric oxide synthase (eNOS). Recent clinical data report that diseased human vessels contain increased levels of the oxidative stress enzyme myeloperoxidase (MPO) and considerable data now supports a pathogenic role for MPO in endothelial dysfunction and heart fibrosis in cardiovascular disease patients. A pre-requisite for MPO to cause endothelial dysfunction is its transcytosis across the endothelium and deposition into the sub-endothelial matrix where the enzyme is anatomically positioned to promote oxidative reactions that impact on endothelial function. Previous studies (Eiserich *et al.*, 2002) indicate that sub-endothelial MPO deposits can promote endothelial dysfunction by catalytically consuming NO. In the presence of chloride ions and hydrogen peroxide (H_2O_2) MPO also forms the potent oxidant hypochlorous acid (HOCl). MPO can also utilize physiological pseudo-substrates nitrite or thiocyanate to yield nitrogen dioxide radical or hypothiocyanous acid (HOSCN), respectively.

Objective: Investigate the implications of these different oxidants produced by endothelial-transcytosed MPO for endothelial function and dysfunction and define the oxidative and molecular signaling reactions involved.

Results and Conclusions: In inflammatory aorta isolated from mice injected with lipopolysaccharide (10 mg/kg) for 4 h we detected increased levels of MPO and HOCl oxidized proteins in the vascular endothelium. Incubation of isolated arteries or cultured endothelial cells (ECs) with MPO resulted in the enzyme's transcytosis through ECs and deposition into the sub-endothelial matrix where it co-localizes with fibronectin fibrils. Exposure of the MPO-containing EC or arteries to low micromolar (<50 μM) concentrations of H_2O_2 afforded increased rates of peroxide consumption that coincided with the production of HOCl and resultant oxidative modification of fibronectin in the sub-endothelial matrix.

In MPO-containing EC, addition of H_2O_2 resulted in a rapid phase (0 - 10 min) of increased eNOS activity that correlated with a coordinated increase in phosphorylation of eNOS at Ser-1177 and de-phosphorylation at Thr-495. Pro-longed exposure (>30 min) of MPO-containing EC with H_2O_2, however, resulted in impaired cellular uptake of the eNOS substrate, L-arginine and reduced eNOS enzyme activity. Pre-treatment of MPO-containing vessels with H_2O_2 impaired endothelial-dependent vessel relaxation in a manner abrogated by inhibition of MPO activity, scavenging of HOCl or addition of thiocyanate or nitrite to divert MPO from HOCl production and instead produce HOSCN or nitrogen dioxide. MPO-containing EC and vessels exposed to H_2O_2 were also characterized by the increased production of superoxide anion radical, the selective removal of which restored vasorelaxation responses to control levels. Together these studies show that despite acutely activating eNOS, HOCl produced by endothelial-transcytosed MPO impairs endothelial function in a superoxide anion radical-dependent manner.

In studies aimed at investigating the implications of MPO-induced oxidative modification of fibronectin for endothelial signalling, morphology and function we found that matrix oxidation triggered the rapid retraction of the cell membrane from both the substratum and from adjacent cells (*i.e.* de-adhesion), quantified in real-time by a cell-substrate impedance biosensor and live cell imaging. EC de-adhesion was attenuated by inhibition of actomyosin contraction using the myosin II inhibitor blebbistatin or the Rho kinase inhibitor Y-27632. Loss of cell-matrix contact caused a rapid Src kinase-dependent phosphorylation/activation of paxillin, a key focal adhesion protein governing 'outside-in' integrin signalling in ECs. Notably, addition of thiocyanate or nitrite to form HOSCN or nitrogen dioxide, inhibited fibronectin oxidation and associated changes in cell signalling and de-adhesion. These studies show endothelial-transcytosed MPO catalyses HOCl-mediated oxidation of sub-endothelial matrix components to trigger focal de-adhesion, myosin II-dependent cell contraction and altered focal adhesion signaling. This novel mode of 'outside-in' redox-dependent signalling is likely to have important implications for the maintenance of endothelial barrier integrity and function during vascular inflammatory conditions where MPO deposition into the sub-endothelial space is a feature.

Eiserich JP, Baldus S, Brennan ML, Ma W, Zhang C, Tousson A, Castro L, Lusis AJ, Nauseef WM, White CR, Freeman BA. (2002) *Science* **296**: 2391-4.