Scientific Paper:

Free Radical Biology & Medicine 42, 1165-1177, 2007

ROS generation in endothelial hypoxia and reoxygenation stimulates MAP kinase signaling and kinase-dependent neutrophil recruitment

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Abstract:

Reactive oxygen species (ROS)-induced injury has been shown to occur during the reperfusion phase of ischemia–reperfusion and ROS are known to induce signaling events. We hypothesized that oxygen sensing in endothelial cells is also dependent on internal redox changes during hypoxia and that endothelial cells respond to changing oxygen environments via signaling, switching to an inflammatory phenotype. Endothelial cells exposed to relative hypoxia or the mitochondrial inhibitors rotenone, antimycin A, or FCCP show loss of mitochondrial membrane potential. During hypoxia, an increase in cytoplasmic ROS and glutathione S-transferase activity occurred, suggesting changes in intracellular redox state, mimicked with rotenone or FCCP but inhibited by antimycin A. Phosphorylation of stress-responsive mitogen-activated protein kinases occurred in hypoxia and was rapid and prolonged. Phosphorylation was inhibited by vitamin C, N-acetyl cysteine, or antimycin A. Chelation of intracellular calcium inhibits phosphorylation but the mitochondrial transition pore inhibitor cyclosporin A had no effect. Reoxygenation caused a further round of signaling, which was rapid but transient. Functionally, adhesion of neutrophils after hypoxia–reoxygenation under flow is ROS, Pselectin and MAPK dependent. Therefore, changes in cellular signaling and phenotype are abrogated by ROS scavengers and suggest their use as therapeutic agents in ischemia–reperfusion.

Key-words: Endothelium, ROS, MAP kinase, ischemia-reperfusion, free radicals