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Hypothiocyanous Acid (HOSCN) Induced Cellular Dysfunction : Evidence for the Inactivation of Intracellular Enzymes

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Myeloperoxidase (MPO) released by activated phagocytes, forms reactive oxidants by catalysing the reaction of halide and pseudo-halide ions with H_2O_2. These oxidants have been linked to tissue damage in a range of inflammatory diseases. With physiological levels of halide and pseudo-halide ions, similar amounts of HOCl (hypochlorous acid) and HOSCN (hypothiocyanous acid) are produced by MPO. Although the importance of HOSCN in initiating cellular damage via thiol oxidation is becoming increasingly recognised, the mechanism(s) involved remain poorly characterised. In this study, the ability of HOSCN to induce the inactivation of various key thiol-dependent intracellular enzymes in macrophage cells was studied. Treatment of the cells with HOSCN resulted in a dose-dependent loss in the activity of glyceraldehyde-3-phosphate dehydrogenase (GAPDH), cathepsin B, cathepsin L and creatine kinase (CK). This loss in activity is attributed to the oxidation of the active thiol site residue in each case, as evidenced by the correlation of loss in activity and rapid thiol oxidation in experiments with the isolated enzymes. In the case of CK and cathepsins B and L, HOSCN induced inhibition at lower oxidant concentrations than HOCl. Similar results were obtained on treating J774A.1 lysates with the MPO/H_2O_2/SCN^- system. In this case, greater inactivation of GAPDH and cathepsins B and L was observed in the presence of SCN^- (to generate HOSCN) compared to Cl^- (to generate HOCl). A significant reduction in cellular ATP levels was also observed on treatment of cells with HOSCN, which is attributed to the inactivation of intracellular enzymes, including CK. This may have important implications for people with elevated levels of SCN^- arising from cigarette smoking, and play a role in the pathologies associated with this biological insult.

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Bioenergetics Changes Induced by Hydrogen Peroxide Exposure in Endothelial Cells

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Increased reactive oxygen species (ROS) generation underlies many pathologies of the cardiovascular system including ischemia/reperfusion injury, atherosclerosis, and the peripheral vascular complications of diabetes. While mitochondrial bioenergetic pathways have been reported to be compromised in these pathologies, little is known about how mitochondria function in response to oxidative stress in intact cells since previous studies were performed using isolated mitochondria. Here we determined the effects of bolus doses of hydrogen peroxide (H_2O_2) on a kinase important in controlling cellular energy metabolism (AMPK) as well as mitochondrial respiration of adherent cells using a Seahorse Bioscience XF24 Extracellular Flux Analyzer. In bovine aortic endothelial cells (BAEC), we found that up to 500 μM H_2O_2 was not cytotoxic, however AMPKα was activated in a dose dependent manner and mitochondrial respiration was altered. We observed an increase of the basal oxygen consumption in a dose-dependent manner in cells treated with H_2O_2 with respect to control cells. In addition, specific mitochondrial parameters were evaluated by sequential addition of inhibitors of the respiratory chain. The maximal and reserve capacities were decreased in the same manner by the addition of the uncoupler FCCP, indicating a loss in maximal respiratory capacity at Complex IV. Taken together these results demonstrate a decrease in the bioenergetics of intact cells by the oxidant, hydrogen peroxide which we hypothesize involves activation of the AMP kinase pathway.

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Mitochondrial Nitration of Fatty Acids

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The levels of nitrated fatty acids (NO_2-FA) are elevated in mitochondria during ischemia-reperfusion. Despite the multiple signaling pathways involving NO_2-FA, the mechanism of formation still remains elusive. Several in vitro reactions using non-physiological conditions demonstrated the relevance of radical reactions involving nitric oxide (NO) and nitrogen dioxide and acidic nitration. Nitrite (NO_2^-) is the main metabolic product of NO and an important mediator of S-nitrosylation. Herein, we describe that isolated mitochondria is the main source of nitrated oleic, linoleic and linolenic acids, a process characterized by its dependence on NO_2^- levels (0.01-10 mM) and pH values (6-7.4). Nitration was dependent on enzymatic activities as demonstrated by the inhibition elicited by azide, cyanide or heat treatments. The mitochondrial nitration resulted in the same NO_2-FA found during cardiac ischemia-reperfusion. Incubation with $^{15}NO_2^-$ resulted in ^{15}N incorporation into mitochondrial NO_2-FA. Exogenously added fatty acids did not impact the formation of nitrated species, suggesting that nitration of complex lipids and phospholipase activities may be involved. NO_2-FA were detected as early as 5 min after NO_2^- addition, and increased over time. These products reacted with nucleophiles (e.g. GSH and β-mercaptoethanol) and were readily reduced to nitroalkanes by sodium borohydride. In addition, the formation of GSH adducts was detected in mitochondria. Moreover, the relative position of the nitroalkene moiety was defined using a modified, highly sensitive, MS-MS lithium adduct-based method. In summary, we demonstrated that mitochondria, via an enzymatic mechanism using NO_2^- as substrate, is a relevant source of NO_2-FA at physiological pH values. These NO_2-FA are electrophilic, react with GSH and protein targets, and directly impact and modulate mitochondrial homeostasis and respiration.

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Influence of Oxidative Stress on the Formation of Advanced Glycation End-Products

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There is substantial evidence that advanced glycation end-products (AGEs) play a major role in the development of complications related to aging, diabetes, and uremia. Our own work and work from others provide growing evidence that oxidative stress is a major promoter for AGE formation *in vitro* and *in vivo*.In order to investigate the role of oxidative stress in the