fluorescence dye, TMRM. Quantitative image analysis showed that the compounds could be grouped into four categories: (a) those that caused superoxide formation and a loss in MMP at similar concentration ranges (for example, astemizole, sorafenib), (b) those that caused superoxide production but had no effect on MMP (dasatinib), (c) those that caused a loss in MMP but had no effect on superoxide formation (tamoxifen, sertraline), and (d) those that had no effect either on superoxide formation or on the MMP over 24 hours (pioglitazone, risperidone). Both high content imaging assays were robust and rapid and can be implemented within a screening paradigm to identify compounds that modulate oxidative stress and mitochondrial membrane potential.

PS 2007 The Antioxidant Lipoic Acid Exacerbates Paraquat-Induced Cytotoxicity.

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In several countries the use of herbicides has become important in the preservation of sustainable agriculture. A widely used herbicide for broadleaf weed control is paraquat (PQ) known to be toxic to humans and animals. The treatment against PQ poisoning remains supportive with no antidotes or specific treatments available. Recognizing that PQ induces its toxicity primarily via oxidative stress-mediated mechanisms, modulating the levels of cellular antioxidants seems to serve as a potential treatment strategy. We studied the in vitro effects of the thiol-containing antioxidant lipoic acid (LA) in a human lung adenocarcinoma epithelial cell line. Incubation of control A549 cells with PQ resulted in time- and concentration-dependent increases in intracellular PQ levels with concomitant decreases in cell viability and mitochondrial membrane permeability (MMP) and increases in intracellular calcium concentrations [Ca+2]i. Challenge of cells with LA alone did not cause any changes in any of the biochemical parameters measured with the exception of the MMP being significantly decreased. Co-treatment of A549 cells with LA and PQ potentiated the PQ-induced cytotoxicity as evidenced by the further exacerbation of PQ-induced decreases in MPP and increases in DNA fragmentation and [Ca+2]i. Chromatographic analysis (GC/MS/MS) showed that LA was primarily associated with cell membranes. These data suggest that LA does not offer any protective effects against PQ-induced toxicity. The mechanism(s) for its ability to modulate cell survival/death by modulating the cellular redox-regulated signal transduction in PQ-challenged cells is under investigation. This research project was supported by Natural Sciences and Engineering Research Council of Canada (NSERC).

PS 2008 Lipid Droplets with Oxygenated Fatty Acids and Triglycerides in Dendritic Cells: Possible Role in Antigen Presentation in Cancer.

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Immuno-surveillance plays a critical role in control of tumor progression whereby dendritic cells (DC) are the most potent antigen presenting cells responsible for the development of immune responses. Our previous work has identified lipid droplets as potent regulators of DC's immune functions. Notably, DCs isolated from tumor-bearing mice or treated with tumor explant supernatants (TES) accumulated lipid droplets containing considerable amounts of PUFA (C18:2, C18:3, C20:4 and C22:6). Among those, the amounts of C18:2 were significantly higher compared to other PUFA species. Since accumulation of lipid droplets in DCs in cancer was mediated via Msr1 receptor, which is primarily responsible for the uptake of oxidatively modified lipids, we performed analysis of oxidized lipids in DCs using LC-ESI-MS. To investigate possible role of oxidized fatty acid in antigen presentation we used a model system were DCs from naïve mice were grown in the presence of C18:2. Treatment of DCs with C18:2 in combination with a hydrophobic free radical generator, an azo-intiator AMVN, - but not with C18:2 alone - resulted in accumulation of oxygenated lipid droplets and caused significant decrease in antigen presentation. Oxygenated FFA containing one, two and three oxygens as well as oxygenated triacylglycerols, TAGs, including truncated TAGs with m/z 766 [M+NH4]+, containing acyl corresponding to 9-oxo-nonanoic acid, were observed in DC grown in the presence of TES and DC treated with C18:2 and AMVN. Given that lipid droplets can directly co-localize with cellular compartments involved in antigen processing and formation of pMHC complexes, it is likely that accumulation of oxygenated FFAs and TAGs in DC may be responsible for the loss of their immune-regulatory functions in cancer. Supported by grant with CA165065, NIOSH OH008282, NIH U19 AI068021, HL70755.

PS 2009 Mitochondrial Cardiolipin As a Substrate for Cytochrome C-Catalyzed Production of Oxygenated Lipid Mediators.

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Lipid mediators – central to the normal homeostasis and responses to stress and disease - are generated through oxygenation of polyunsaturated fatty acids (PUFA), such as linoleic acid (LA), arachidonic acid (AA) and docosahexaenoic acid. Their regulatory effects are believed to depend on a fine balance between PUFA esterification/reacylation of phospholipids and on their hydrolysis by phospholipases A (PLA). We suggested that mitochondrial cardiolipins (CLs) can be a source of bioactive lipid mediators generated with the catalytic participation of cytochrome c (cyt c). In this study we employed models of rat traumatic brain injury (TBI) and mousetotal body irradiation (TIR). Using oxidative lipidomics approach and MS/MS analysis, we found that TBI resulted in oxidation of polyunsaturated molecular species of CL and accumulation of its hydrolysis products such as oxygenated LA, AA and monolyso-CL. Similar, a significant increase of oxidized CLs in small intestine of TIR mice (9.5 Gy) was accompanied by accumulation of CL hydrolysis products. To generate oxygenated CL in vitro we utilized brain CL, with its highly diversified polyunsaturated molecular species, and cyt c. We found that incubation of brain CL with cyt c in the presence of H2O2 yields a rich assortment of oxygenated CL species, hydrolysis of which by PLA1 and A2 generated multiple oxygenated fatty acids similar to those that were formed in vivo in brain after TBI and small intestine after TIR. An oxidation-specific lipoprotein lipase A2, was able to utilize peroxidized tetralinoleoy-CL to yield different oxygenated species of linoleic acid and lyso-CLs. Thus, mitochondrial CL/cyt c represents a novel mechanisms involved in lipid mediators-generating pathways. Supported by NIH ES020693, ES021068, U19 AI068021, HL70755; NIOSH OH008282, NS076511, NS061817.

PS 2010 Development of a Mitochondria-Targeted Nano-Complex of Imidazole-Substituted Oleic Acid As a Radiomitigator.

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Increasing likelihood of intended or accidental exposure to ionizing radiation dictatesthe necessity to develop effective medical countermeasures of radiation injury as has been recognized as a high priority both in the US and worldwide. No effective medical radiation countermeasures of acute and delayed radiation injuries are currently known. Based on newly discovered mechanisms of radiation damage – oxidation of cardiolipin by cytochrome c in mitochondria as a required stage in radiation-induced apoptosis - we designed and synthesized mitochondria-targeted triphenylphosphonium-conjugated imidazole-substituted oleic (TPP-OA) which prevented/mitigated cell death induced by irradiation and protected C57BL6 mice against total body irradiation. To improve therapeutic efficiency of TPP-IOA we chose to employ branched polyethylene glycol (PEG) functionalized single walled carbon nanotubes (SWCNT) and use it as a carrier to deliver mitochondria-targeted TPP-IOA to tissues. We found that loading of PEG-SWCNT with TPP-IOA caused a marked extension of the life-span of TPP-IOA in circulation. Moreover we showed that TPP-IOA-nano-complex was more effective as radiomitigator than free TPP-IOA. While the dose of TPP-IOA in TPP-IOA-nano-complexes was two times lower compared with free TPP-IOA the mitigating effect of TPP-IOA-nano-complexes was higher than that of TPP-IOA alone. Importantly, we were able to detect TPP-IOA nano-complexes in radiosensitive tissue such as small intestine. These data warrant further studies aimed at the development of radioprotectors/radiomitigators with broad spectrum of applications in biomedicine and biodefense. Supported by NIOSH OH008282; NIH U19 AI068021, HL70755, ES019304.

PS 2011 XBP1, SYVN1 and Nrf2: At the Crossroads of ER Stress and Oxidative Stress.

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Transcription Factor Nrf2 has long time been revealed as the master regulator of intracellular redox homeostasis. As an adaptive response to oxidative stress, Nrf2 actives transcription of a battery of genes encoding antioxidant protein, detoxification

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