PS 1180 2D-LC-MS ANALYSIS OF OXIDIZED LIPID SPECIES: APPLICATION TO THE STUDY OF CARDIOLIPIN IN APOPTOSIS AND DAMAGED TISSUE.

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Oxidized lipid species are important signaling molecules that usually exist in low abundance in biological tissues. Along with their inherent stability issues, these oxidized lipids present themselves as a challenge in their detection and identification. These oxidized lipid species can co-chromatograph with non-oxidized species making their detection extremely difficult. In this study, a normal-phase and reverse-phase two dimensional HPLC-mass spectrometric system was applied to separate oxidized phospholipids from their non-oxidized counterparts, allowing unambiguous detection in a total lipid extract. We have utilized bovine heart cardiolipin as well as commercially available tetralinoleoyl cardiolipin oxidized with cytochrome c and hydrogen peroxide as well as with lipoxygenase to test the separation power of the system. We found that oxidized and nonoxidized phospholipid species can be clearly separated from in this two dimensional system. We utilized two biologically relevant model systems, namely rotenone treatment of lymphocytes to induce mitochondrial damage and cell death as well as traumatic brain injury, to separate and identify cardiolipin oxidation products, critical to the cell death pathways in these tissues following cellular stress/injury. We identified oxidized linoleic acyl chains (50%) as the preferred moieties for oxidation in cardiolipin, followed by arachidonic (31%) and docosahexaenoic acyl chains (18%). The ability of the two-dimensional HPLC-mass spectrometric system to detect and characterize oxidized lipid products will allow new studies to be formulated to probe the answers to biologically important questions with regard to oxidative lipidomics and cellular insult. Supported by NIOSH OH008282; NIH U19 AI068021, HL70755, HL094488, NS061817; ES020693, ES021068.

PS 1181 CELL-BASED ANALYSIS OF LIPID PEROXIDATION AND LIPID PEROXIDATION-DERIVED PROTEIN MODIFICATIONS USING FLUORESCENCE MICROSCOPY.

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Oxidative stress plays an important role in the progression of several diseases including inflammation, atherosclerosis, aging and age-related degenerative disorders. Reactive oxygen species damage membrane bound lipids including unsaturated fatty acids like linoleic acid and arachidonic acid to form lipid electrophiles, which can rapidly react with proteins and DNA to form adducts. Cell-based measurements of lipid peroxidation and protein carbonylation by fluorescence microscopy provide a powerful platform to quantitate lipid peorxidation in cells and also monitor spatial distribution of damage caused by lipid peroxides. Here, we used 2 different approaches to measure lipid peroxidation in cells. 1) A ratiometric determination of lipid peroxidation using a fluorescent ratiometric lipid probe for live cells which is incorporated into cellular membranes and emits at 590 nm. Under oxidative stress conditions, the oxidation of the dye results in a shift of fluorescence to a peak emission of 510 nm. 2) In a click chemistry based approach, unsaturated fatty acid analogs like linoleic acid alkyne or arachidonic acid azide are incorporated into the cellular membranes and the products resulting from oxidation, like HNE and DODE can readily modify DNA or proteins. The modified proteins are then analyzed by click reaction using fluorescent tagged alkynes or azides. Using these approaches, we measured lipid peroxidation caused by oxidants like tert-butyl hydroperoxide, cumene hydroperoxide, menadione, hemin and lipolysaccharide in BPAE and RAW macrophage cells. The oxidants produced 2-3 fold increase in lipid peroxidation and protein modifications when compared to controls. The lipid peroxidation and the lipid peroxide-derived protein modifications were successfully inhibited by using anti-oxidants like α-tocopherol and mixed tocopherols. The 2 strategies described here to measure lipid peroxidation and the derived protein modifications provide powerful tools to measure oxidative stress in cells.

PS 1182 MALDI MASS SPECTROMETRIC IMAGING OF CARDIOLIPIN AND ITS OXIDATION PRODUCTS DIRECTLY FROM TISSUE SECTIONS IN A LUNG OXIDATIVE DAMAGE MODEL.

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Molecular understanding of lipid functions and signaling mechanisms has created the need for tissue selective assessment for localization of phospholipids and their oxidation products while maintaining tissue integrity. Cardiolipin (CL) is a mitochondria-specific anionic phospholipid that plays key roles in normal mitochondrial functions but also in cell death pathways. The molecular diversity of endogenous CL in some tissues and the low abundance of its individual species, particularly oxidized derivatives, makes in situ analysis challenging. Current Mass Spectrometry protocols using lung homogenates (LC-MS) have greatly enhanced our ability to explore molecular species of CL and its oxidized products. However location-specific information on oxidation cannot be determined from homogenates. Assuming that oxidative lipidomics analysis of acute lung injury will directly benefit from MALDI-MS imaging, we assessed possible advantages of this frontier technology in characterization of structural lipid changes in the lung. MALDI-MS imaging was performed on thin lung sections from both naïve mice and ones receiving glucose oxidase (GOX) intratracheally as a model of oxidative injury. Scanning was at the diameter of the laser beam (20 microns), and each location scanned generated a complete mass spectrum that correlated with tissue location. A strong increase in oxidative degradation products of CL was observed by MALDI-MS imaging. Light microscopy confirms the preservation of tissue structure (without fixation). Serial sections were homogenized and analyzed by LC-MS to confirm the CL species identified by MALDI-MS Imaging. Assignment to an anatomical region suspected to be injured (e.g. airways) has been achieved. Supported by NIOSH OH008282; NIH U19 AI068021, HL70755, HL094488, ES020693, ES021068, CCSG-P30 CA047904

PS 1183 GLUTATHIONE DYNAMICS AND DIFFERENTIAL SENSITIVITY TO PRO-OXIDANTS DURING ZEBRAFISH DEVELOPMENT.

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The embryonic development of vertebrates is highly susceptible to disruption by exposure to chemicals, many of which may cause oxidative stress. However, very little is known about the endogenous oxidative stress response and antioxidant defenses during early life stages. We have characterized the developmental redox dynamics of the most abundant endogenous antioxidant defense system, glutathione. We measured total glutathione, including reduced (GSH) and oxidized (GSSG) species, and calculated the whole embryo reduction potential over the first 120 hours of zebrafish development. Total glutathione concentrations doubled between 12 hours post fertilization (hpf) and hatching. The glutathione redox potential (Eh) became less negative during the first 12 hours and then remained at approximately -190 mV until a rapid change at hatching to a highly negative (more reducing) Eh (-220 mV). This Eh remained steady through 120 hpf. We then examined the impact of these changes in glutathione concentrations and redox potential on the differential sensitivity of embryos to pro-oxidant exposure at different developmental stages. The sensitivity of embryos to a 4-hr exposure to the model pro-oxidant tert butyl hydroquinone (tBHQ) decreased prior to hatching, with embryos exposed at 48 hpf being the least sensitive; the glutathione redox dynamics were surprisingly resilient with these exposures. After hatching (between 72 and 120 hpf), embryos became increasingly more sensitive to the lethal effects of tBHQ and another pro-oxidant, tert butyl hydroperoxide (tBOOH). This differential sensitivity is not explained by the changes in either glutathione concentration or reduction potential alone. However, these factors together, they may define the cellular redox dynamics important for activation of the oxidant-responsive transcription factor Nrf2 and subsequent upregulation of antioxidant defenses, and thus impact oxidant sensitivity. [F32ES017585, R01ES015912, R01ES016366]

PS 1184 THE EFFECT OF MENADIONE ON GSTA1-JNK COMPLEX DISSOCIATION AND JNK ACTIVATION IN CACO-2 CELLS.

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Glutathione-S-transferases (GSTs) are a family of phase II detoxification enzymes that catalyze the conjugation of glutathione (GSH) to a variety of environmentally and endogenously produced electrophilic substances. The human alpha class GSTA1 is critical in the cellular defense against the deleterious effects of oxidative stress through their selenium-independent peroxidase activity. GSTs also act as modulators of MAPK signal transduction pathways via a mechanism involving protein–protein interactions. We have demonstrated that GSTA1 forms complexes with JNK. However the factors that regulate the dissociation of GSTA1 from JNK have not been identified. We hypothesized that menadione-mediated oxidative stress activates JNK, causes dissociation of GSTA1-JNK complexes and that the degree of oxidative stress depends on the level of GSTA1 expression. Human colonic adenocarcinoma (Caco-2) cells show a progressive increase in GSTA1 protein ex-

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