PS 2450 ROTENONE-INDUCED OXIDIZED MOLECULAR SPECIES OF CARDIOLIPIN IN HUMAN LYMPHOCYTES: CAN THEY BE USED AS BIOMARKERS OF MITOCHONDRIAL DYSFUNCTION ASSOCIATED WITH PARKINSON'S DISEASE?

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During the last three decades, epidemiological and toxicological studies have provided data that pesticides, including rotenone, contribute to the development of Parkinson's disease (PD). Circulating lymphocytes are often used to study the pathogenic mechanisms in PD. We reasoned that oxidized molecular species of CL in human lymphocytes will progressively accumulate in the course of exposure to rotenone. We isolated lymphocytes from human blood obtained from the Central Blood Bank. Using an oxidative lipidomics approach based on 2D-LC/ESI-MS, we found that CL in human lymphocytes was represented mainly by readily oxidizable species containing polyunsaturated fatty acid residues. The contents of CL molecular species with polyunsaturated fatty acid residues - C18:2, C20:4 and C22:6 - were as high as 67.7%, 24.7% and 5.4%, respectively. Only a small amount (2.2% of total CL) of non-oxidizable CL molecular species containing C18:1 was found in human lymphocytes. Detailed structural characterization of oxygenated CL molecular species in lymphocytes treated with rotenone (250 μM, for 18h) revealed the presence of oxidized CL molecular species. Their characterization demonstrated that oxidized CL was represented by a profile containing 13 different hydroxy- and hydroperoxy-molecular species. Further studies of specificity of rotenone induced CL oxidation in rat midbrain and correlations with the unique stereo-specific molecular species of CL formed in mitochondria of human and rat lymphocytes in response to rotenone exposure will determine the extent to which these profiles of lymphocyte CL oxidation may lead to reliable biomarkers of mitochondrial dysfunction associated with neurodegenerative disorders. Supported by NIOSH OH008282; NIH U19 AI068021, HL70755, HL094488, ES020693.

PS 2451 DETERMINATION OF ENDOGENOUS AND EXOGENOUS ACETALDEHYDE-DERIVED DNA ADDUCTS.

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Acetaldehyde (AA) is a ubiquitous chemical that is produced endogenously during normal metabolic activity; it is ethanol's primary metabolite and is used in a wide variety of industrial activities. AA is a known animal carcinogen, causing squamous cell carcinomas and adenocarcinomas in nasal tissue of rats and laryngeal carcinomas in hamsters following inhalation exposures. AA is highly reactive and can adduct to both proteins and DNA forming DNA adducts and DNA-protein cross-links. N^2-Ethyldiene-dG is the primary DNA adduct formed following AA exposure to DNA; while stable in DNA, it is unstable following digestion to nucleosides. To address the instability, DNA was reduced using $NaCNBH_3$ to convert N^2-ethyldiene-dG to the more stable N^2-ethyl-dG adduct. As AA exposures are a result of endogenous and exogenous AA, a sensitive and selective method combining HPLC fraction collection, followed by detection and quantitation using nano-LC-MS/MS was developed and validated. DNA was extracted, reduced with $NaCNBH_3$, and enzymatically digested prior to HPLC fractionation and nano-LC-MS/MS analysis. The limit of detection was 10 amol on column and the limit of quantitation was 20 amol on column. The method was validated with accuracy, precision, recovery, and matrix effects assessed. Endogenous N^2-ethyl-dG was quantitated at 3.5 ± 0.1 adducts per 10^7 dG in calf thymus DNA. This method will be used to determine endogenous and exogenous N^2-ethyl-dG adducts following [$^{13}C_2$]-AA exposure in cell culture. TK6 human lymphoblast cells (~2 x 10^7 cells/exposure) were exposed to [$^{13}C_2$]-AA for 12 hours at concentrations ranging from 0.00005 – 2.0 mM AA, along with negative controls. Following completion of the exposure, the media was removed, cells washed and frozen at -80°C prior to DNA extraction. The method will be used to determine the exposure-response relationships between endogenous and exogenous AA-DNA adducts following [$^{13}C_2$]-AA exposure.

PS 2452 BIOMARKERS AND ONSET OF NONSPECIFIC BUILDING-RELATED SYMPTOMS IN THE DWELLING: A COHORT STUDY FROM 1992 TO 2002.

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We examined associations between biomarkers of allergy and inflammation, indoor environment in dwellings and incidence and remission of symptoms included in the sick building syndrome (SBS) and changes of the home environment in 452 adults followed from 1992 to 2002 within the Uppsala part of the European Community Respiratory Health Survey (ECRHS). The 10-year incidence (onset) of general, mucosal, and dermal symptoms was 12.7%, 25%, and 7.7%, respectively. Dampness or indoor moulds at baseline was a predictor of incidence of general (RR=1.98), mucosal (RR=2.28) and dermal symptoms (RR=1.91). Females had higher incidence of general (RR=1.74) and mucosal symptoms (RR=1.71). Indoor painting increased incidence of general symptoms (RR=1.62). Bronchial hyperresponsiveness (BHR), eosinophil counts in blood, total IgE and eosinophilic cationic protein (ECP) in serum at baseline were predictors of incidence of SBS. At follow up, BHR; total IgE and C-reactive protein (HCRP) were associated with increased incidence of SBS. Moreover, subjects with doctors' diagnosed asthma at baseline had a higher incidence of general symptoms (RR=1.65) and mucosal symptoms (RR=1.97). In conclusion, female gender, dampness or indoor moulds, indoor painting and biomarkers of allergy and inflammation were associated with a higher incidence of SBS symptoms, in particular mucosal symptoms. The association between incidence of SBS symptoms and clinical biomarkers of allergy and inflammation suggests a common aetiology between inflammatory diseases such as asthma and rhinitis and SBS.

PS 2453 VALIDATION OF A NONANTIBODY METHOD FOR DETECTION OF SERUM AFLATOXIN B1-LYSINE ADDUCT IN FISCHER 344 RATS.

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Serum aflatoxins B1-lysine adduct (AFB-lys) is a validated biomarker for studying human AF exposure. Recently we have developed a non-antibody based method for rapid detection of serum AFB-lys adducts in human populations in developing world. To further validate this method, male Fischer 344 rats were orally administered AFB via different dosing regimens and serum AFB-lys levels were assessed using this method. The single dosing regimen (SDR) included a control, 50, 250, and 1000μg AFB/kg body weight groups and blood was collected at 2h, 1, 3, 5, and 7 days post treatment. The repeated dosing regimen (RDR) included a control, 5, 10, 25, and 75μg AFB/kg body weight groups and blood was collected at 1, 2, 3, 4, and 5 weeks. Serum was prepared and AFB-lys was analyzed. In the SDR, AFB-lys adduct was detectable at the maximal level at 2h post treatment with 1.68 ± 0.22 ng AFB-lys/mg albumin in 50μg AFB/kg group, followed by 61% (0.66 ± 0.05), 65% (0.58±0.12), 83% (0.28±0.02), and 92% (0.14 ±0.02) decreases in 1, 3, 5, and 7 days, respectively. Similar pattern was found for 250 and 1000μg AFB/kg groups. In the RDR, the prolonged exposure for 5-week increased serum AFB-lys levels to 0.54 ± 0.05, 1.06 ± 0.08, and 3.00 ±0.20 ng AFB-lys/mg albumin at 5, 10, and 25μg AFB/kg groups, respectively. However, the maximal level of AFB-lys (9.06 ± 0.84 ng AFB-lys/mg albumin) was found at 2-week after repeated exposure to 75μg AFB/kg, followed by a 4% (8.67 ±0.90), 12% (7.96 ±0.31), and 22% (7.06 ±0.43) decreases at 3-, 4-, and 5-week, respectively. These results indicated either saturation of the adduct level with exposure to this dose or impaired liver function of enzymatic activation of AFB in test animals. Excellent correlations between AFB administrated and AFB-lys adduct level were found. Taken together, results of this study demonstrate the validity of our method for detecting serum AFB-lys adduct.

PS 2454 IDENTIFICATION AND CHARACTERIZATION OF INTRACELLULAR PROTEINS IN LNCAP CELLS EXPOSED TO BIZ-2.

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Prostate cancer is the leading cause of death in men in the United States. Currently, there are no known cures for this health problem. Kola Nut (Cola acuminata) or Bizzy Nut is a plant that contains androgenic-type hormonal activity. Work from

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