932 3β-HYDROXY-5-OXO-5, 6-SECOCHOLESTAN-6-AL, A MAJOR OZONIZATION PRODUCT OF CHOLESTEROL, INDUCES APOPTOSIS IN CARDIOMYOCYTES.

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Ozone toxicity induces pulmonary dysfunction in humans and animals. Since ozone does not penetrate far (<2 μ) into the lung parenchyma, the adverse effects of ozone are thought to be mediated via the ozonation products formed at the air/lung interface. Thus, phospholipids, cholesterol, and cholesterol esters present in the epithelial lining fluids and in the alveolar cell membranes are among the primary targets for the reaction of ozone. The interaction of ozone with these lipids results in the formation of a number of toxic metabolites including 3β-hydroxy-5-oxo-5, 6-secocholestan-6-al (Chol-Seco), the major ozonation product of cholesterol. Most studies to date have concentrated on the effects of ozone and the ozonation products on the lung parenchyma. The present study was undertaken with the purpose of determining the extra-pulmonary effects of ozone mediated via cholesterol ozonation using cardiomyocytes derived from the embryonic rat myocardium (H9C2 cells). The H9C2 cells were treated with varying low concentrations (0-10 μM) of Chol-Seco for 12-24 h at 37±0.5 °C. After incubation, the cells were fixed and analyzed using flow cytometry and immunocytochemistry. It was observed that Chol-Seco induces a time and dose dependent increases in cardiomyocyte apoptosis. The lowest dose of 2 μM Chol-Seco for instance, was found to cause 14±1.1% apoptosis against DMSO controls that only showed 3±0.1% apoptosis. At 5 and 10 μM Chol-Seco, the percent apoptotic cells at 24 h were 45±3.8 and 52±2.0, respectively. Apoptosis was also seen at 12 h and 18 h, with the highest dose inducing greater cell death. Anexin staining showed a similar pattern. These results suggest that cholesterol ozonation product(s) is/are potent inducers of apoptosis in cardiomyocytes and might play a role in ozone induced myocardial injury. (Funding support from NIEHS ES10018 and LBRN is acknowledged.

933 INCREASED SUSCEPTIBILITY OF HYPERTHYROID RATS TO OZONE: EARLY EVENTS AND MECHANISMS.

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Previous studies have determined that ozone-induced lung damage and inflammation are much greater in hyperthyroid vs. normal rats at 18 hours following exposure. The purpose of the present investigation was to study early events and mechanisms underlying the increased sensitivity to ozone in a hyperthyroid state. Specifically, the degree of lung epithelial cell barrier disruption, extracellular lining fluid antioxidant status, and the release of inflammatory mediators were examined. To create a hyperthyroid condition, mature male Sprague-Dawley rats were implanted with time-release pellets containing thyroxine; control rats received placebo pellets. After 7 days, the animals were exposed to air or ozone (2 ppm, 3 h). Immediately following the end of the exposure, bronchoalveolar lavage (BAL) fluid and cells were harvested. BAL fluid albumin levels and total antioxidant status were examined. In addition, levels of PGE2, MIP-2, MCP-1, and TNF-α were determined in BAL fluid and following *ex vivo* culture of BAL cells. The results of this study are consistent with the following hypotheses: 1) a marked increase in the permeability of the alveolar-capillary barrier is an early event underlying the increased susceptibility of hyperthyroid rats to ozone, however this does not appear to be due to overall changes in BAL fluid antioxidant potential; 2) early increases in MIP-2, but not PGE2, are involved in the enhanced lung response to ozone in a hyperthyroid state; 3) inflammatory mediator production (i.e., PGE2, MIP-2, MCP-1, and TNF-α) by alveolar macrophages plays a minimal role in the initial responses to ozone in a hyperthyroid state.

934 INHIBITION OF TRANSFORMING GROWTH FACTOR BETA MNRA TRANSCRIPTION BY ANTISENSE OLIGONUCLEOTIDES IN AMIODARONE TREATED HAMSTERS.

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Pulmonary fibrosis is an often fatal disease characterized by cellular hyperplasia and excessive accumulation of extracellular matrix components with remodeling of the lung. The precise series of events leading to such scarring are not well understood. Amiodarone is an antiarrhythmic drug that is known to cause pulmonary fibrosis. Amiodarone induces an increase in transforming growth factor beta (TGFβ) and such increase plays a significant role in the fibrotic process. The hypothesis of the current study is that blocking TGFβ mRNA transcription will modulate amiodarone induced pulmonary fibrosis in hamsters. Golden Syrian hamsters were treated with TGFβ antisense oligonucleotides by aerosolization. Following such treatment animals were exposed to amiodarone by intratracheal insufflation. The hamsters were euthanized 24 hours after treatment, bronchoalveolar lavage (BAL) samples obtained and the lung tissue either frozen in liquid nitrogen or fixed in formalin and embedded in paraffin. TGFβ levels were measured in BAL samples by Western blot analysis. TGFβ levels were significantly decreased in animals treated with the antisense oligonucleotides as compared to control. BAL samples from treated and control animals were analyzed by light microscopy to determine the level of inflammatory cells present in the lung. A significant decrease was observed in the number of inflammatory cells present in the BAL fluid from antisense treated animals as compared to controls. Immunohistochemical analysis of frozen tissue sections from animals treated with antisense oligonucleotides prior to toxicant exposure exhibited a decrease in TGFβ levels as compared to controls. Morphologic study revealed reduction in areas of interstitial thickening in animals treated with TGFβ antisense oligonucleotides as compared to the controls. Antisense oligonucleotides to TGFβ mRNA inhibited, but did not completely prevent cytokine production in animals exposed to amiodarone and reduced alterations in lung architecture.

935 INVESTIGATION OF PREVENTION OF AMIODARONE- AND DESETHYLAMIODARONE-INDUCED TOXICITY IN HUMAN PERIPHERAL LUNG EPITHELIAL CELLS HPL1A.

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Amiodarone (AM) is a highly effective antidysrhythmic used in the treatment of serious ventricular and supraventricular dysrhythmias. The mechanisms underlying AM-induced pulmonary toxicity (AIPT) have not been elucidated. Evidence suggests an important role for N-desethylamiodarone (DEA), a major AM metabolite, in AIPT. The immortalized, non-transformed human peripheral lung epithelial cell line (HPL1A) is morphologically and biochemically very similar to normal human peripheral lung epithelial cells. HPL1A cells have many characteristics that make them excellent candidates for assessing the mechanisms of toxicant action in human peripheral lung epithelial cells. In this study, HPL1A cells were grown to approximately 80% monolayer confluence, transferred to 96-well plates and incubated with AM or DEA concentrations varying from 0 to 1 mM. At 2, 4, 8, 12, 24, and 36 hours, viability was assessed using the 3-[4, 5-dimethylthiazol-2-yl]-2, 5-diphenyltetrazolium bromide (MTT) assay. Time- and concentration-dependent losses of cell viability were observed, with DEA being more cytotoxic than AM at shorter incubation times. AM toxicity, expressed as the concentration that produces a 50% loss of cell viability, drastically increased with the incubation time. In contrast, DEA toxicity showed little variation over the incubation time period. Prior incubation with the spin trap a-phenyl-N-t-butylnitrone (PBN, 10 mM) for 30 minutes provided only slight protection against AM/DEA-induced toxicity. These results support that long-term rather than acute exposure of HPL1A cells to AM is associated with an increased loss in cell viability. The observed cytoprotective effect of the spin trap suggest that AIPT may be in part mediated by a free-radical process. HPL1A cells represent a potentially competent model for elucidating the mechanisms of the AM-induced adverse pulmonary effects in humans. (Supported by Canadian Institutes of Health Research grant number MOP-13257).

936 GENE MICROARRAY ANALYSIS IN A RAT MODEL OF SMOKE INHALATION-INDUCED ACUTE LUNG INJURY.

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In a rat model of acute lung injury (ALI)/acute respiratory distress syndrome (ARDS)-like injury, we examined 28, 757 genes cDNA microarrays for early transcriptome alterations in the homolized lung tissue at 24 hours post-smoke insult. Data indicated that genes responsible for membrane enzyme, transporter, hemoglobin, signal transducer, binding activity, and transcription regulation were dysregulated. Smoke inhalation-mediated alterations in the identified genes were largely sorted into many signaling transduction pathways, including chemokine/cytokine, hormone, DNA binding, transcription factor, and G-protein receptor. Moreover, genes encoding different oxidoreductase, transferase, and isomerase were affected. Among them, the rapid inductions of sulfotransferase (Sult-n) and inositol/phosphatidylinositol kinase (Pik3cb) suggest that the metabolism of smoke toxins were prominent in the acute phase of smoke inhalation-induced ALI/ARDS. These findings reveal that there are complex molecular cascades involving disturbances in different subcellular compartments, influencing thereafter the normal cellular functions, leading to ALI/ARDS (Supported by ALA).

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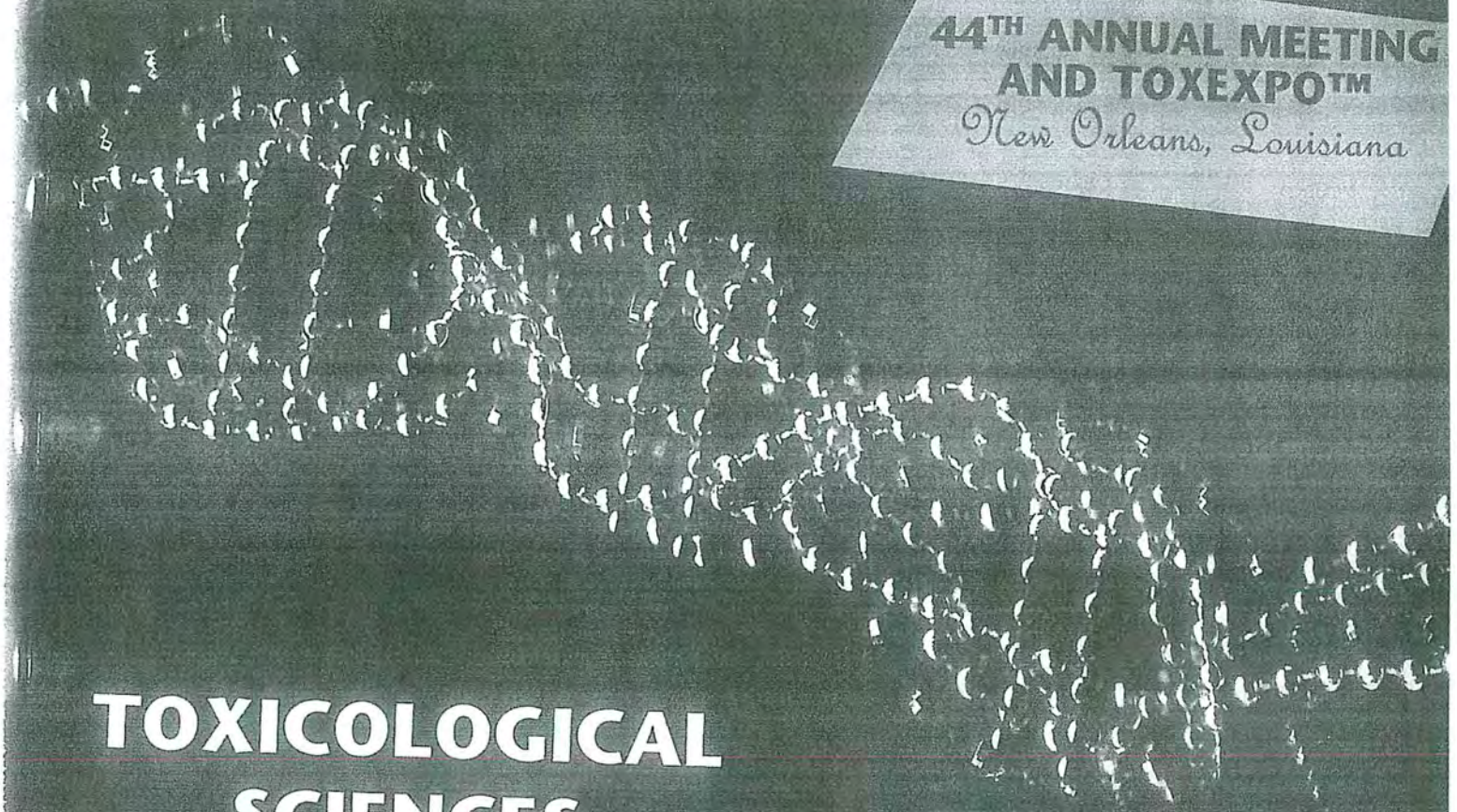

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