PS 936 OXIDATIVE LIPIDOMICS OF MACROPHAGE ACTIVATION AND APOPTOSIS INDUCED BY PHAGOCYTOSIS OF PARTICLES AND PATHOGENS.

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Macrophages are essential to the innate immune system in eliminating invading microorganisms and as major orchestrators of the inflammatory response. As pathogens undergo phagocytosis and intracellular digestion; this mechanism can lead to apoptosis of the phagocyte. However, the mechanisms of apoptosis and its links with phagocytosis are largely unknown. We hypothesized that triggering of phagocytosis and activation of macrophages – including NADPH oxidase generated derived reactive oxygen species - mediates selective oxidation of phospholipids participating in the execution of apoptotic program. To approach this, zymosan, silica particles and carbon nanotubes were utilized as different types of pathogens/particles capable of activating macrophage in vitro and/or in vivo. Macrophage cell lines (RAW 264.7 and IC-21) as well as C57Bl/6 mice were treated with one of the above and NADPH oxidase activation, superoxide generation, cytochrome c release, PS externalization and caspase 3/7 activity were used to confirm the apoptotic cell death. Oxidation of major classes of phospholipids - phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylserine (PS), phosphatidylinositol (PI), and cardiolipin (CL) - was determined by electro-spray ionization mass spectrometry as well as by fluorescence HPLC/Amplex Red assay. The results demonstrated that robust and selective peroxidation of anionic phospholipids (CL>> PS >PI) accompanied macrophage apoptosis in vitro. Moreover, oxidation of the same species of anionic phospholpipids was detected in lungs of mice exposed to single walled carbon nanotubes via pharengeal inhalation. We conclude that anionic phospholipid-mediated signaling may participate in phagocytosis induced macrophage apoptosis. Supported by NIOSH OH008282, NORA 927000Y, NIH HL70755, ES010859 and the 7th Framework Program of the European Commission.

PS 937 SIRT1 IS POST-TRANSLATIONALLY MODIFIED BY ALDEHYDES AND CIGARETTE SMOKE IN LUNG EPITHELIAL CELLS.

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Rationale: Sirtuin1 (SIRT1), a class III histone/protein deacetylase, is involved in stress responses and regulation of inflammation, apoptosis and aging. We have previously shown that SIRT1 protein levels are decreased in lungs of smokers and patients with COPD leading to increased NF-κB-dependent inflammation. As oxidative modification of proteins can alter both a protein's function and level, we hypothesized that oxidative modification of SIRT1 by cigarette smoke (CS) and oxidants leads to down regulation of SIRT1 activity and protein level. ***Methods:*** Human bronchial (BEAS-2B) and primary airway epithelial cells were exposed to different concentrations of cigarette smoke extract (CSE 0.5-1%), H_2O_2 (150 μM), or acrolein (10 and 30 μM) for 6 hours. Lysates were used to measure SIRT1 activity (*Color de Lys assay*, Biomol) and oxidative modifications using biotin switch assay. ***Results:*** SIRT1 activity was significantly decreased by CSE, acrolein, and H_2O_2. However, only CSE and acrolein dose-dependently decreased SIRT1 levels. Pre-treatment with N-acetyl-L-cysteine (2 mM) attenuated CSE depletion of SIRT1 levels and activity. Labeling cell lysates with maleimide-biotin revealed that SIRT1 was modified on cysteine residues in response to CSE, and that NAC could prevent these modifications. Treatment of cells with N-ethylmaleimide (100 μM) decreased SIRT1 activity as well as protein levels further implicating modification of cysteines in SIRT1 regulation. Furthermore, SIRT1 is degraded by proteasome as proteasomal inhibitors (5 μM ALLN and 10 μM MG-132) attenuated CSE-mediated reduction of SIRT1. ***Conclusions:*** Post-translational modification of SIRT1 by carbonylation on cysteine residues inactivates the enzyme and renders it for proteasomal degradation. These data may have implications for stress responses and abnormal inflammation in response to environmental stimuli and oxidants.

PS 938 IMPACT OF PEROXIREDOXIN 6 GENE DELETION AND OVER-EXPRESSION ON ETHANOL-MEDIATED LIVER DAMAGE IN MICE.

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Oxidative stress is implicated in the etiology of many diseases including alcoholic liver disease (ALD). Peroxiredoxin 6 is a cytosolic peroxidase that has been demonstrated to protect various tissues, such as skin, lung and cardiac muscle, against acute oxidative insults. Taken together, peroxiredoxin 6 was hypothesized to also protect the liver from oxidative stress generated during the process of chronic ethanol metabolism. To test this, both peroxiredoxin 6 knockout mice (KO) and transgenic, peroxiredoxin 6 over-expressing mice (TG) were fed an ethanol containing diet and various biomarkers of ALD were assessed along with the effects of chronic ethanol consumption on the antioxidant defenses. After 9 weeks of ethanol consumption all backgrounds exhibited increased plasma ALT activity, steatosis, CYP2E1 induction and lipid peroxidation. Differences in antioxidant protein expression and activity were also observed. Significantly induced catalase and glutathione S-transferase activity in ethanol-fed KO and TG mice along with elevated levels of glutathione peroxidase activity were noted. These results could be attributed to either compensatory responses due to the genetic manipulations or ethanol-mediated responses. It can then be concluded that both ethanol-fed KO and ethanol-fed TG mice developed early stage ALD and that oxidative stress and overall pathology were not exacerbated by the absence of peroxiredoxin 6 and not prevented or attenuated by its over-expression.

PS 939 OXIDATIVE STRESS IS THE MECHANISM OF PPAR-INDUCED SKELETAL MYOPATHY IN RATS.

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The PPAR-alpha receptor is activated by fibrates which causes proliferation of peroxisomes, hepatomegaly and hepatocarcinogenesis in rodents. Skeletal muscle is also a major site of PPAR-alpha and delta expression and represents a principal tissue responsible for lipid uptake and utilization. Skeletal myopathy, and rhabdomyolysis have been reported with administration of fibrates. The aim of this study was to determine the mechanism by which PPAR (alpha/delta) agonists induce skeletal myopathy in rats. Sprague Dawley rats were treated with GW610742X, a dual pharmacologic agonist of PPAR (alpha/delta) nuclear receptors, over a time course study with animals sacrificed at 6 hrs, 24 hrs, 2, 4, 6, and 10 days. The soleus muscle, extensor digitorum longus muscle, heart, liver, whole blood and serum were collected at each time point. In addition to standard clinical chemistry and histopathology, samples were analyzed using transcriptomic (Affymetrix) and metabolomic (Fourier transform mass spectroscopy) techniques. Minimal skeletal myopathy was first noted in soleus (type I) muscle of some animals on Day 4, with the lesions becoming progressively more severe and homogeneous over the remainder of the time course. Development of skeletal myopathy was preceded by hepatic peroxisome proliferation, increased plasmalogens (antioxidant lipids) and lipid peroxides in the serum, and decreased GSH in liver, whole blood and skeletal muscle. The onset of myopathy was also preceded by increased fatty acid beta-oxidation in the soleus, and was coincident with a strong induction of genes associated with oxidative stress. Based on these data, we propose that PPAR-induced skeletal myopathy in rats is the result of a combination of two pharmacological effects; hepatic peroxisome proliferation which results in systemic GSH deficiency, and increased beta-oxidation in Type I skeletal muscle. Together, these effects result in oxidative stress which leads to the observed skeletal myopathy.

PS 940 INDICATORS OF OXIDATIVE STRESS AND APOPTOSIS FOLLOWING EXPOSURE TO STYRENE AND ITS METABOLITES IN MOUSE WHOLE LUNG AND CLARA CELLS.

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Styrene causes both acute and chronic toxicity in humans. In mice, styrene is both hepato- and pneumo-toxic and causes lung tumors. One possible explanation for the tumorigenicity is oxidative stress/damage. Decreases in glutathione levels, linked to increases in apoptosis, occur in lung homogenates and isolated Clara cells 3 hrs following styrene or styrene oxide (SO) administration. Since low levels of reactive oxygen species (ROS) have been linked with increased apoptosis, ROS levels following administration of styrene and its metabolites were measured in vitro and in vivo. Both in vitro and in vivo measurements showed significant increases in ROS 3 hrs after styrene, R-SO, S-SO, and racemic SO administration. None were seen with styrene or R-SO at 12 hrs or beyond. Ratios of bax/bcl-2 mRNA expression were determined at time periods from 3 to 240 hrs following exposure to styrene and R-SO. The mRNA expression ratio increased at 12 and 24 hrs following R-SO and at 120 hrs following styrene administration. Since a consequence of oxidative stress can be 8-hydroxydeoxyguanosine (8OHdG) adduct formation, levels were measured in mouse lung homogenates 3 hrs and 12 hrs after the administration of 600 mg/kg styrene or 300 mg/kg R-SO. Significant increases in 8OHdG with both compounds were seen at 3 hrs, but not at 12 hrs. Thus both ROS and

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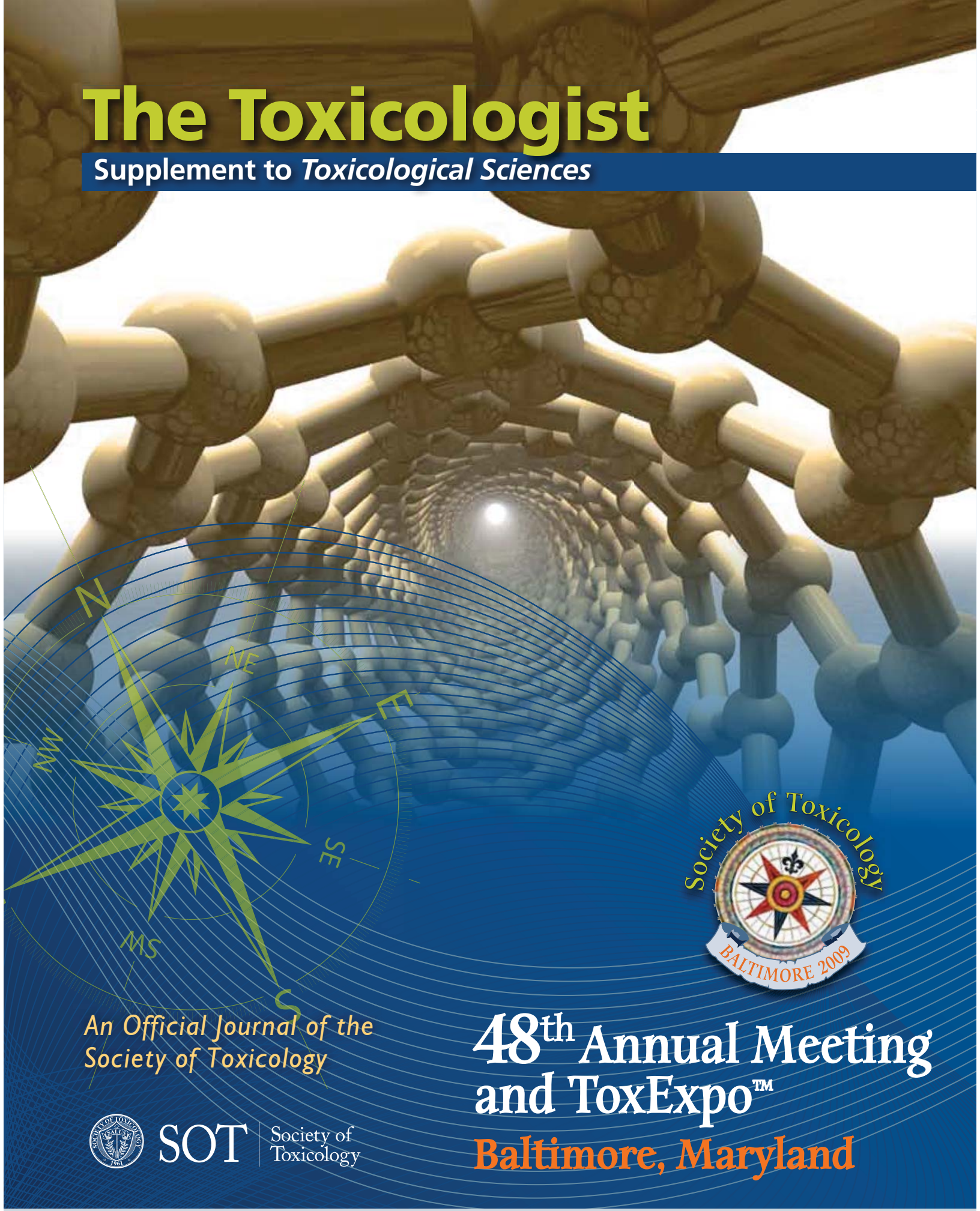

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