activities of the respiratory enzymes, aconitase, NADH dehydrogenase and cytochrome c oxidase. The treated HepG2 cells undergo apoptosis triggered by the release of cytochrome c from the mitochondria and activation of caspases. In addition, the expression of antiapoptotic protein Bcl2, as well as iNOS and COX-2, was also inhibited in aspirin treated cells. Furthermore, we have also demonstrated that the GSH pool and the activities of the GSH-metabolising enzymes, glutathione S-transferase and GSH-peroxidase were increased and GSH-reductase was decreased in these cells. Some of the key mitochondrial functions affected by aspirin appear to be associated with the alterations in GSH pool as buthionine sulfoximine (BSO), a GSH depleting agent, treatment has augmented its effects and the supplementation of N-acetylcysteine has attenuated the effects of BSO. Our results strongly suggest that the anticancer effects of aspirin on cancer cells might be associated with mitochondrial dysfunction and oxidative stress.

1396 CARBACHOL PREVENTS OXIDATIVE STRESS-MEDIATED APOPTOSIS INDUCED BY DOMOIC ACID IN CEREBELLAR GRANULE CELLS.

G. Giordano[1], L. Li[1], T. Kavanagh[1] and L. Costa[1,2]. *[1]Environmental and Occupational Health Sciences, University of Washington, Seattle, WA and [2]Human Anatomy, Pharmacology and Forensic Science, University of Parma Medical School, Parma, Italy.*

In mouse cerebellar granule neurons (CGNs) low concentration of domoic acid induce apoptotic neuronal cell death, which involves caspase-3 activation and is mediated by oxidative stress. The cholinergic agonist carbachol caused a concentration-dependent inhibition of domoic acid-induced apoptosis, which was antagonized by atropine and by the M3 muscarinic receptor antagonist 4-diphenylacetoxyl-N-methylpiperidine methiodide. Carbachol also inhibited domoic-acid-induced activation of caspase-3 and of p38 and JNK kinases. Carbachol did not prevent domoic acid-induced elevation in ROS, suggesting that the antiapoptotic effect of carbachol occurs downstream of ROS production. Carbachol induced phosphorylation of Akt and of Erk 1/2, without altering the level of expression of unphosphorylated protein.
The protective effect of carbachol was reversed by specific inhibitors of pro-survival intracellular signaling proteins, including an inhibitor of phosphatidylinositol 3-kinase (LY294002), of Akt (Akt inhibitor III) and of MEK/MAPK (UO126).
It has been shown that activation of the PI3K/Akt pathway can prevent oxidative stress-mediated apoptosis through the transcription-dependent elevation in glutamate-cysteine ligase (GCL) activity, which is the rate limiting step in the synthesis of glutathione (GSH). Carbachol caused a time –dependent increase of GCL activity and of GSH levels. Both effects were antagonized by inhibitors of the PI3K/Akt pathway, but not by the inhibitor of the MEK/MAPK pathway. This increase in GCL activity occurred without any change in the level of expression of the catalytic and modifier subunits. These results indicate that stimulation of muscarinic receptor activates the PI3K/Akt pathway leading to increase of GCL activity. The resultant increase in intracellular GSH levels restores the cellular redox balance antagonizing the oxidative stress-mediated apoptosis induced by domoic acid. Supported by ES012762/NSF-OCE-0434087.

1397 THE EFFECTS OF JS-K AND PABA/NO, NOVEL ANTI-TUMOR NITRIC OXIDE RELEASING PRODRUGS, IN HUMAN HEPATOMA HEPB3 CELLS.

J. Liu[1], Q. Wu[2], L. K. Keefer[3], J. E. Saavedra[4] and M. Waalkes[1]. *[1]ICS, LCC, NCI at NIEHS, Research Triangle Park, NC, [2]Zunyi Medical College, Zunyi, China, [3]CS, LCC, NCI at Frederick, Frederick, MD and [4]SAIC, NCI at Frederick, Frederick, MD.*

The O^2-arylated diazeniumdiolates JS-K and PABA/NO are nitric oxide (NO) releasing prodrugs that have been shown to have anticancer activity in a variety of model systems, including human HL-60 leukemia cells, multiple myeloma cells, prostate cancer PPC-1 cells, ovarian A2780 cancer cells, and JM-1 hepatoma cells. To expand these findings, the present study examined effects of JS-K and PABA/NO in human hepatoma HepB3 cells. HepB3 cells were treated with various concentrations of JS-K or PABA/NO, and cytolethality was determined 48 hrs later by the MTS assay. The LC_{50} values for JS-K was about 15 μM, and for PABA/NO 20 μM, while the specific chemical compound controls lacking the NO moiety (JS-43-126 for JS-K and JS-39-93 for PABA/NO) showed no apparent cytotolethality at levels up to 100 μM. At well tolerated concentrations (3-10 μM), both JS-K and PABA/NO potentiated the cytolethality produced by sodium arsenite, an emerging chemotherapeutic metalloid, and the LC_{50} for arsenite was shifted from 20 μM to about 10 μM. To examine gene expression changes associated with JS-K and PABA/NO induced tumor cell death, HepB3 cells were treated with 0-10 μM JS-K or PABA/NO for various times (12 - 48 hrs), and total RNA was isolated, purified, and subjected to real-time RT-PCR analysis. JS-K and PABA/NO treatment induced changes in gene expression typical for cell death and growth arrest. These included increased expression of genes associated with apoptosis (*caspase 3* and *caspase 8*), growth arrest (*p53*), acute phase response (*c-jun* and *IL-1β*), anti-angiogenesis such as ribonuclease/angiogenin inhibitor, *RA1*. Both NO releasing compounds also altered various genes related to tumor cell invasion such as tissue inhibitors of metalloproteinases (*TIMP1* and *TIMP2*). Thus, multiple molecular events are likely associated with JS-K and PABA/NO modulated cell death in human HepB3 hepatoma cells, and may contribute to an overall antitumor effect.

1398 GOLD NANOPARTICLES IN RATS AFTER INHALATION EXPOSURE.

L. E. Yu[2], W. Ong[1], L. Yung[3], C. Ong[4], Y. Tan[1], K. Balasubramanian[2], D. Hartono[3], G. Shui[5] and M. R. Wenk[5]. *[1]Anatomy, National University of Singapore, Singapore, Singapore, [2]Environmental Science & Engineering, National University of Singapore, Singapore, Singapore, [3]Chemical & Biomolecular Engineering, National University of Singapore, Singapore, Singapore, [4]Community Occupational and Family Medicine, National University of Singapore, Singapore, Singapore and [5]Biochemistry, National University of Singapore, Singapore, Singapore.* Sponsor: U. Boelsterli.

Gold nanoparticles (nAu) are used in consumer products, suggesting potential exposure to this material during manufacturing process or use of consumer products. Since gold in bulk form is chemically inert compared to other metallic materials, any biologic effects of nAu are likely due to factors other than intrinsic toxicity of the material. To systematically assess potential health effects of engineered nanomaterials, inhalation exposure was carried out to investigate the distribution of gold in various organs of rats. Possible changes in gene expression of the lung / kidney were also examined.
Adult male Wistar rats were exposed to airborne nano-sized particles (NSPs) (2 x 10^6 #NSPs/cm3) for 6 hours per day for 5 or 15 days. Accumulated gold in tissue samples were quantified using ICP-MS.
After 5-day exposure, significant accumulation of gold was detected in the lung (100 ng Au/g tissue) and olfactory bulb (8 ng Au/g tissue). After 15-day exposure, significant accumulation of Au was detected in over 20 organ and tissues. Of the above tissues, the highest concentrations of gold were substantially observed in lung, esophagus, tongue, kidney, aorta, spleen, septum, heart and blood. In the lung, genes that showed the greatest downregulation (>10 folds) were related to muscle. The lung and kidney showed significant downregulation in more than two genes. Expression of the gene for thyroid hormone responsive protein in the kidney was also significantly decreased.
Inhaled nAu is able to translocate from the lung to other organs with time, and can cause significant genetic changes in exposed tissues. Findings of this work pave ways to study toxicity mechanisms and effects of function of inhaled nAu on targeted organs.

1399 USE OF LABELED SINGLE WALLED CARBON NANOTUBES TO STUDY ACUTE TRANSLOCATION FROM THE LUNGS.

R. R. Mercer, J. F. Scabilloni, L. Wang, L. A. Battelli and V. Castranova. *HELD, National Institute for Occupational Safety and Health, Morgantown, WV.*

Single walled carbon nanotubes (SWCNT) have a small size and unique physical-chemical properties which suggest that novel particles may translocate to systemic organs after deposition in the lungs. In this study, gold particles (10nm) were used to label a highly dispersed preparation of SWCNT with a mean geometric diameter of 1.1 microns. The gold labeled SWCNT were delivered to the lungs by pharyngeal aspiration to C57BL/6 mice. Neutron activation analysis (NAA) of lung, blood and other organs was carried out at various times after aspiration to determine if the gold labeled SWCNT translocated out of the lungs. Six mice per group were studied at 1 hour, 1 day and 3 days after exposure to a single dose of 50 μg of gold labeled SWCNT. A separate group was given gold particles without SWCNT. A phosphate buffered saline (PBS) aspiration group served as the negative control. At sacrifice the lungs, heart, brain, liver, kidneys and a blood sample were taken for analysis of gold content by NAA. Gold content of the PBS aspiration group was negligible. In the gold labeled SWCNT group lung gold content at 1 day was 65 percent of that at 1 hour and not significantly different from the content at 3 days. The heart, brain, liver, kidney and blood samples from the gold labeled SWCNT groups contained less than 1 part in 200 of the total dose and were not statistically different from the PBS aspiration group at 1 hour, 1 day and 3 days. The results indicate that SWCNT do not readily translocate from the lungs.

SOT | Society of Toxicology

48th Annual Meeting and ToxExpo™

Baltimore, Maryland

March 15–19, 2009

Baltimore Convention Center

Photos courtesy of Baltimore Area Convention and Visitors Association

47th Annual Meeting and ToxExpo™

March 16–20, 2008

SOCIETY OF TOXICOLOGY 2008 SEATTLE

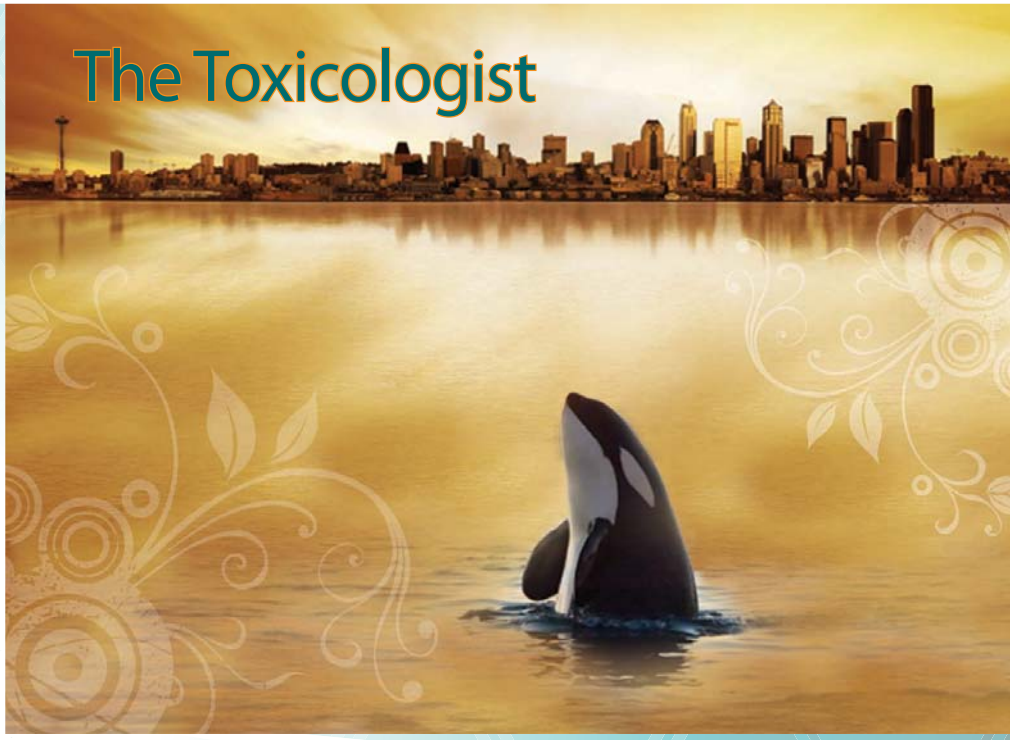
Society of Toxicology
1821 Michael Faraday Drive, Suite 300 • Reston, VA 20190
T: (703) 438-3115 • F: (703) 438-3113 • E-mail: sothq@toxicology.org
www.toxicology.org

SOT | Society of Toxicology

The Toxicologist

March 2008

The Toxicologist



SOCIETY OF TOXICOLOGY 2008 SEATTLE

47th Annual Meeting and ToxExpo™

Seattle, Washington

Supplement to Toxicological Sciences
An Official Journal of the Society of Toxicology

SOT | Society of Toxicology

www.toxsci.oxfordjournals.org
OXFORD UNIVERSITY PRESS ISSN 1096-6080 Volume 102, Number 1, March 2008