556 OXIDATIVE STRESS IN SILICOSIS: KINETIC CLEARANCE ASSAY OF TEMPO RADICALS.

V Vallyathan, S Leonard, P Kuppusamy, D Pack, M Zhahan, S P Sanders and J Zweir. *NIOSH, Morgantown, WV and The Johns Hopkins Medical Institutions, Baltimore. MD.* Sponsor: V Castranova.

A sensitive and specific kinetic clearance assay using low frequency electron spin resonance (ESR) has been developed to facilitate investigations of the pathophysiological role of oxidative stress in lungs. Using this technique we investigated ROS' dependent kinetic clearance of instilled stable nitroxide radicals (TEMPO) in silicosis. Superoxide dismutase (SOD), glutathione peroxidase (GPx), and lipid peroxidation were also studied in whole lungs of rats exposed to silica (quartz) and controls. The clearance of TEMPO followed first order kinetics showing significant differences in the rate for clearance between quartz vs sham exposed control lungs. Comparison of TEMPO clearance rates in the sham exposed controls and silicotic rats indicated an oxidative stress in the rats exposed to quartz. Studies of antioxidant enzymes SOD and GPx, in the lungs of quartz and sham exposed animals supported the oxidative stress and accelerated clearance of TEMPO by up regulated levels of enzymes in quartz exposed animals. Increased lipid peroxidation potential in the silicotics also supported a role for enhanced generation of ROS. These *in vivo* experiments directly demonstrate that silicotic lungs are in a state of oxidative stress and that increased generation of ROS is associated with enhanced levels of oxidative enzymes and lipid peroxidation. This technique offers great promise for the elucidation of ROS induced lung injury and development of therapeutic strategies for the prevention of damage.

557 FORMATION OF 8-OXOGUANINE FROM THE *IN VITRO* UVA IRRADIATION OF CALF-THYMUS DNA AND QUINOLONES.

T E Spratt, D Chen, G Schlüter, and G M Williams. *American Health Foundation, Valhalla, NY and Bayer AG, Wuppertal, Germany.*

Several quinolone antibiotics have been shown to produce oxidative DNA damage in rat liver cells in culture following the administration of UVA. To begin our characterization of the chemical mechanism of this observation we first wanted to determine if the formation of 8-oxo-2'-deoxyguanosine (8-oxo-dG) was dependent on metabolism of the hepatocytes. To this end, DNA and quinolone were incubated in the presence of UVA and the amount of 8-oxo-dG in the DNA was determined. Calf-thymus DNA was incubated with quinolones and irradiated with 1 J/cm^2 of UVA. The DNA was precipitated away from the excess quinolone. Following hydrolysis with Nuclease P1 and alkaline phosphatase the amounts of dG and 8-oxo-dG was determined by reverse-phase HPLC with UV and electrochemical detection. Four quinolones were tested. The relative amounts of 8-oxo-dG produced in these in vitro reactions were similar to that observed in the cell culture reactions: Bayer Y3118 > lomefloxacin > ciprofloxacin > Bayer 12-8039. The more phototoxic compounds were more photodegraded and produced more 8-oxo-dG. The formation of 8-oxo-dG appears to saturate at high concentrations of Y3118. The amount of 8-oxo-dG found from the irradiation of 12-8039 was not above the background for the concentrations tested.

558 INHIBITION OF HYPOXIA-REOXYGENATION INJURY IN THE HEART OF CATALASE OVEREXPRESSING TRANSGENIC MICE.

Y Chen, [1] J T Saari[2] and Y J Kang.[1] [1]*Departments of Medicine and Pharmacology and Toxicology, University of Louisville School of Medicine, Louisville, KY and* [2]*U.S. Department of Agriculture, Grand Forks Human Nutrition Research Center, Grand Forks, ND.*

Oxygen-derived free radicals play important roles in hypoxia-reoxygenation or ischemia-reperfusion injury in the heart. Recent studies in isolated heart muscle and whole heart have strongly suggested that H_2O_2 is the primary free radical species responsible for postischemic myocardial damage. However, contradictory reports have been published on the possible effect of catalase on cardiac hypoxia-reoxygenation lesion although catalase is a major enzyme involved in detoxification of H_2O_2. The present study was undertaken to determine whether catalase overexpression in the heart of transgenic mice can provide protection against hypoxia-reoxygenation injury. Transgenic mice with elevated cardiac catalase 60-fold higher than normal were selected and the effects of catalase elevation on H_2O_2 and hypoxia-reoxygenation induced cardiotoxicity were determined by measuring functional and morphological changes in isolated atria. Catalase overexpression suppressed H_2O_2 and hypoxia-reoxygenation reduced contractile force and contraction rate, and eliminated reoxygenation-induced arrhythmia. The catalase overexpressing atria were also highly resistant to hypoxia-reoxygenation induced morphological alterations as examined by electron microscopy. The results thus demonstrate that catalase inhibits hypoxia-reoxygenation injury likely by the function of detoxifying H_2O_2. Supported in part by NIH Grant CA86125.

559 IN VIVO RADIOPROTECTIVE EFFECTS OF OLTIPRAZ: THE ROLE OF ENHANCED GLUTATHIONE S-TRANSFERASE (GST) EXPRESSION.

S G Kim, and S Y Nam. *College of Pharmacy, Duksung Women's University, Seoul, Korea.*

Pretreatment of mice with oltipraz (100mg/kg/d, 2d) prior to lethal dose of γ-rays irradiation (8 Gy) substantially increased the 30 day-survival rate. Previous studies showed that radiation in combination with oltipraz enhances hepatic epoxide hydrolase expression. The effects of γ-rays irradiation in combination with oltipraz on the expression of hepatic GST subunits Ya, Yb1/2 and Yc1/2 were examined in the rat. Northern blot analysis revealed that GST gene expression was altered in response to daily 3 or 0.5 Gy irradiation. Whereas the hepatic GST Ya, Yb1/2 and Yc1/2 mRNA levels were transiently decreased at 3 to 8 h after a single 3 Gy irradiation, the GST mRNA levels were increased by 2- to 4-fold at 15 to 24 h post-irradiation, followed by returning to those of control at 48 h time point. Whereas oltipraz at the dose of 30mg/kg caused a 2-fold elevation in the hepatic GST Ya mRNA level, exposure of animals to both oltipraz and 3 Gy radiation resulted in a 4-fold relative increase, indicating that the Ya mRNA expression was additively enhanced by the combination treatment. The radiation-inducible Yb1/2 and Yc1/2 mRNA expression was also enhanced by oltipraz. Multiple exposure of rats to daily 0.5 Gy radiation caused time-related increases in GST gene expression. The greatest enhancement in GST expression was observed at 24 h after a single 0.5 Gy radiation in conjunction with oltipraz (e.g. a 9-fold increase in GST Ya), whereas the relative additive increases in GST mRNA were less pronounced at day 3 to 5 post-treatment. Histopathological examinations revealed that exposure of rats to radiation (0.5 Gy/d, 3-5d) caused moderate hepatocyte degeneration with sinusoidal congestion whereas oltipraz effectively blocked the radiation-induced liver injury. Enhanced expression of the GST genes by oltipraz may be associated with its hepatoprotective effect against radiation injury.

560 CALCIUM MEDIATES ONSET OF THE MITOCHONDRIAL PERMEABILITY TRANSITION DURING OXIDATIVE STRESS TO RAT HEPATOCYTES.

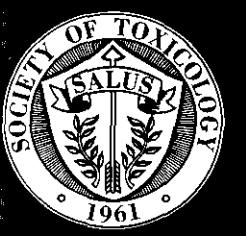
A- L Nieminen[1], A M Byrne[1], and J J Lemasters[2]. [1]*Department of Anatomy, Case Western Reserve University and* [2]*Department of Cell Biology & Anatomy, University of North Carolina, Chapel Hill.*

Previously, we showed that the oxidant chemical, *tert*-butylhydroperoxide (*t*-BuOOH), induces a mitochondrial permeability transition (MPT) in intact hepatocytes leading to cell death (*Biochem. J.* 307, 99). Here, we investigated the role of intracellular free Ca^{2+} in this model of injury. Cultured hepatocytes were exposed to 100 μM *t*-BuOOH. Cell viability, mitochondrial free Ca^{2+}, onset of the MPT and $\Delta\Psi$ were monitored by confocal microscopy. *t*-BuOOH caused an increase in mitochondrial free Ca^{2+}, followed by the onset of MPT, collapse of $\Delta\Psi$ and loss of cell viability. *t*-BuOOH caused also cell killing in calcium-free buffer containing 0.5 mM EGTA. Preincubation with thapsigargin (1 μM) to deplete the intracellular calcium stores in the endoplasmic reticulum also did not protect against *t*-BuOOH toxicity in either the presence or absence of EGTA. However, preincubation with BAPTA AM (1–100 μM), a chelator of intracellular free calcium, prevented the MPT and protected against *t*-BuOOH toxicity in a dose dependent manner. Quin-2 AM (1–100 μM), a calcium indicator, also protected against cell death in a dose dependent manner. Ruthenium red (25 μM), an inhibitor of the mitochondrial Ca^{2+} uniporter, protected partially against cell killing. In conclusion, our results suggest that mitochondrial Ca^{2+} uptake mediates onset of the MPT in hepatocytes during oxidative injury, causing lethal cell injury.

561 SENSITIVITY OF NORMAL HUMAN HEPATOCYTES TO REACTIVE OXYGEN AND NITROGEN.

S M D' Ambrosio[1], and F M Robertson[2]. *Departments of* [1]*Radiology and* [2]*Medical Microbiology, The Ohio State University, Columbus, OH.*

Chronic exposure of human liver to hepatotoxins, infectious agents, and chemicals which perturb normal cellular redox pathways increases the formation of intracellular reactive oxygen and nitrogen. If unchecked, these reactive intermediates can lead to cellular and genotoxic damages, resulting in cell



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Preface

This issue of *The Toxicologist* is devoted to the abstracts of the presentations for the symposium, platform, poster / discussion, workshops, roundtables, and poster sessions of the 36th Annual Meeting of the Society of Toxicology, held at the Cincinnati Convention Center, Cincinnati, Ohio, March 9-13, 1997.

An alphabetical Author Index, cross referencing the corresponding abstract number(s), begins on page 371.

The issue also contains a Keyword Index (by subject or chemical) of all the presentations, beginning on page 395.

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