574 SIGNAL TRANSDUCTION PATHWAYS IN CADMIUM-MEDIATED METALLOTHIONEIN-1 EXPRESSION.

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The purpose of this study was to investigate signal transduction pathways that participate in cadmium-induced regulation of metallothionein (MT), a thiol-rich metal binding protein with free radical scavenging properties. Using quantitative real-time RT-PCR, we show that *in vitro* cadmium (Cd) treatment of alveolar epithelial cells causes both time- and dose-dependent changes in steady state levels of MT-1. The expression of MT-1 was significantly elevated above control (25-fold) 4 hours after the addition of 10 μM $CdCl_2$. A maximum increase in MT gene expression (~ 100-fold) was achieved by hours. Transcript levels remained above control at 16 h (30-fold) and eventually returned to baseline levels by 24 hours. Exposure of cultures to 5, 10, or 20 μM $CdCl_2$ for 8 hours resulted in increases of MT expression of 25-, 100-, and 210-fold, respectively. Inhibitors, specific to protein kinase C (PKC), protein kinase A (PKA), ERK kinase (MEK), p38 mitogen activated protein kinase (MAPK), and tyrosine kinases, were subsequently tested to determine how each affected Cd-mediated induction of MT-1. In these experiments, alveolar epithelial cell cultures were pre-treated for 30 min with each inhibitor prior to an 8 hour treatment with 10 μM $CdCl_2$. Induction of MT by Cd was reduced by 70% when PKC activity was suppressed with the inhibitor GF190203X. In contrast, inhibition of MEK with PD90859 resulted in a 50% increase in Cd-mediated MT gene expression. Inhibitors of p38 MAPK and tyrosine kinase caused less dramatic changes in MT mRNA. In conclusion, the results of this study indicate that signal transduction pathways that include PKC and MEK as active components are of importance in the induction of MT-1 by Cd.

575 ANTISENSE-BCL-2-INDUCED SELECTIVE OXIDATION OF PHOSPHATIDYLSERINE IN NCI-H226 CARCINOMA CELLS: ROLE IN APOPTOTIC SIGNALING.

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The major function attributed to Bcl-2 is its ability to confer resistance against apoptosis. We have previously shown that Bcl-2 was effective in preventing of membrane phospholipid oxidation during apoptosis induced by a number of oxidants as well as non-oxidative stimuli. In particular, phosphatidylserine (PS) was protected against apoptosis-associated peroxidation and subsequent PS-dependent signaling events such as PS externalization. To further investigate the antioxidant/anti-apoptotic role played by Bcl-2 in oxidation of phosphatidylserine during apoptosis, we decreased the expression of Bcl-2 in the lung squamous carcinoma cell line NCI-H226 using a synthetic phosphorothioate oligonucleotide targeted against bcl-2 mRNA (antisense-bcl-2). NCI-H226 cells (106) were incubated with media only or with either the antisense-bcl-2 (0.3 M) oligonucleotide or with a specificity/cytotoxicity nonsense (0.3 M) oligonucleotide control for 5 h at 37C. Incubation of NCI-H226 cells in the presence of the antisense-bcl-2 oligonucleotide resulted in a significantly increased amount of apoptotic cells as compared to controls. Using our technique to assess oxidative stress using cis-parinaric acid (PnA), we found a significant oxidation of Pn-A-PS in apoptotic Bcl-2-depleted NCI-H226 carcinoma cells. No significant differences in oxidation of PnA-labeled phosphatidylcholine and phosphatidylethanolamine between control cells and cells after treatment with both nonsense and antisense-bcl-2 was found. This selective oxidation of PS cannot be attributed to changes of phospholipid composition as our HPTLC analysis of the major phospholipid classes showed no differences in the phospholipid composition between control (untreated) cells and cells incubated in the presence of either antisence-bcl-2 and nonsense oligonucleotides. We conclude that Bcl-2 plays an important role in regulation of programmed cell death and PS oxidation during apoptosis.

576 ROLE OF APOPTOSIS INDUCTION IN DEVELOPMENT OF LUNG INJURY FROM HIGH BURDENS OF TITANIUM DIOXIDE.

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We previously examined the role of apoptosis in lung pathology due to silica, a highly toxic particle and demonstrated that apoptotic cells and degradation products significantly accumulate as injury develops. In this study we test the hypothesis that titanium dioxide (TiO2), a particle with no known specific toxicity, induces apoptosis and accumulation of apoptotic degradation products. To test this, male, F344 rats were given intratracheal instillations (IT) of 0.4, 4 and 40 mg of TiO2. At 1 day, 1 week and 4 weeks after instillation rats were sacrificed, lungs preserved by intra-tracheal instillation of fixative. Sections were processed for TdT labeling of apoptotic nuclei with a fluorescent indicator and counter-stained to label non-apoptotic nuclei. The number of apoptotic cell nuclei per lung was measured by morphometric methods. Additional sections were stained with Sirius Red to detect areas of collagen accumulation. Apoptotic cells in the saline instilled lungs were minimal at 1 and 4 weeks (0.2±0.2 and 0.3±0.4 million cells per lung, mean±SE). One week after IT, apoptotic cells per lung were 1.9±0.1, 4.4±0.4 and 13.3±2 million in the 0.4 mg, 4 and 40 mg groups respectively. At 4 weeks the number of apoptotic cells was increased to 5.2±0.5, 9.5±2.0 and 24.1±3.1 million in the 0.4, 4 and 40 mg TiO2 groups. Sirius Red staining of lung sections demonstrated areas with significant connective tissue accumulations in response to the IT of TiO2 in both the 4 and 40 mg TiO2 groups at 4 weeks of exposure. Sirius Red staining of collagen in saline and 0.4 mg TiO2 groups was normal at 1 and 4 weeks. Our results demonstrate that high levels of inert, non-toxic dusts produce significant numbers of apoptotic cells and products. At high levels of lung burden the induction of apoptosis is associated with development of fibrotic foci. The results suggest that injury from the accumulated apoptotic products may be responsible for injury observed in high lung burden exposures to particulates such as TiO2 which have no specific toxicity.

577 BENZO(A)PYRENE 7, 8-DIHYDRODIOL IS GLUCURONIDATED BY HUMAN UGT1A9 AND CAUSES APOPTOSIS IN HEPG2 CELLS.

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The human UDP-glucuronosyltransferases (UGTs) are involved in cellular detoxification and have been implicated as a mechanism to promote genoprotection. Polycyclic aromatic hydrocarbons such as BENZO(A)PYRENE (BP) are metabolized through oxidation and epoxidation to simple and complex phenols. Both phenolic and dihydrodiol metabolites have been shown to be proximate mutagens capable of being activated to ultimate forms. While most of the simple phenols are substrates for glucuronidation, little is known about the more complex dihydrodiols. Using expressed human UGTs (1A1, 1A3, 1A4, 1A6, 1A7, 1A8, 1A9, 1A10, 2B4 and 2B7), we have demonstrated that BP-*trans*-7, 8-dihydrodiol (±) (7, 8-diol) is actively glucuronidated by UGT1A9. In cells deficient in UGT1A9, such as HepG2 cells, experiments were conducted to examine the mutagenic properties of 7, 8-diol on cell viability. Annexin V analysis revealed in a dose dependent fashion that HepG2 cells were undergoing apoptosis following treatment with various concentrations of the 7, 8-diol. DNA fragmentation assays confirmed the presence of internucleosomal cleavage products consistent with the analysis of poly (ADP-ribose) polymerase (PARP) as shown by the appearance of the PARP 89 kD fragment. In Western blot analysis, HepG2 cells treated with 2.5 μM 7, 8-diol led to a two fold increase in cytosolic Cytochrome C, consistent with one of the properties of apoptosis. $Bcl\text{-}_{XL}$, an anti-apoptotic protein, decreased over 50%, while Bax, a pro-apoptotic protein, increased to 150% of that following vehicle treatment. In addition, an extensive down-regulation of xIAP (inhibitors of apoptosis) was detected which paralleled the appearance of apoptotic DNA fragments. In conclusion, HepG2 cells may be a sensitive biological tool to examine the actions of mutagens on apoptosis in addition to monitoring the genoprotective role of the human UGTs on cell viability. (Supported by USPHS grant CA79834)

578 INVOLVEMENT OF OXIDATIVE STRESS IN SDZ IMM 125-INDUCED APOPTOSIS IN RAT HEPATOCYTES.

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The mechanisms underlying the apoptotic activity of the immunosuppressive drug cyclosporine A and its hepatotoxic O-hydroxyethyl-D(Ser)8-cyclosporine derivative SDZ IMM125 in rat hepatocytes are not fully understood. It was the purpose of the present study to investigate the role of oxidative stress in SDZ IMM125-induced apoptosis, and to evaluate the role of caspase-3 by coincubation of anti- and pro-oxidants with rat hepatocytes. SDZ IMM 125 induced a dose-dependent increase in chromatin condensation and fragmentation, and the activation of caspase-3. Supplementing the cell cultures with the antioxidants, DL--tocopherol-polyethylene-glycol-1000-succinate and ascorbic acid significantly inhibited the SDZ IMM125-mediated increase in chromatin condensation and fragmentation, and caspase-3 activity. The reducing agent, dithiothreitol, significantly inhibited the SDZ IMM125-mediated increase in chromatin condensation and caspase-3 activity. The glutathione synthetase inhibitor, buthionine sulfoximine, significantly enhanced SDZ IMM125-mediated caspase-3 activation and increased SDZ IMM 125-mediated chromatin condensation and fragmentation. The present data suggest that SDZ IMM125-induced apoptosis is mediated by the imbalance of anti- and pro-oxidant steady state in rat hepatocytes and that the intracellular redox-state can act as a modulator of apoptosis. The results further suggest that antioxidants and pro-oxidants might act on apoptosis by modifying caspase-3 activity.

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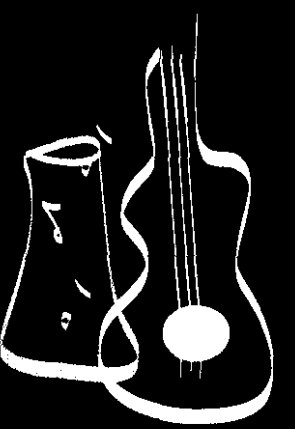

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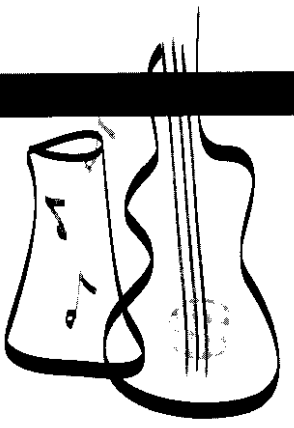

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Preface

This issue of *The Toxicologist* is devoted to the abstracts of the presentations for the symposium, platform, poster discussion, workshop, roundtable, and poster sessions of the 41st Annual Meeting of the Society of Toxicology, held at the Opryland Hotel and Convention Center, Nashville, Tennessee, March 17–21, 2002.

An alphabetical Author Index, cross referencing the corresponding abstract number(s), begins on page 385.

The issue also contains a Keyword Index (by subject or chemical) of all the presentations, beginning on page 411.

The abstracts are reproduced as accepted by the Program Committee of the Society of Toxicology and appear in numerical sequence.

Additional Late-Breaking Abstracts are issued in a supplement to this publication and are available at the 41st Annual Meeting and through the Society of Toxicology Headquarters office.

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