tumor with higher expression in the poorly differentiated tumors. Previously we have shown that the normal human prostate epithelial cell line, RWPE-1, faithfully recapitulates the expression and metal response profile of metallothioinein in normal prostate tissue and can serve as a model system of prostate epithelium. In the course of these studies it was discovered that MT-1E was highly expressed and very inducible with zinc and cadmium. It was hypothesized that this isoform could be highly expressed not only in MT containing tumors but also in tumors lacking the detection of this protein. To assess this possibility, formalin-fixed paraffin-embedded prostate biopsy specimens were assessed for MT protein expression by immunostaining sections with the metallothionein E9 monoclonal antibody. Of these, four metallothionein positive and four metallothionein negative prostate carcinomas that were moderately to poorly differentiated were selected for laser-capture microdissection. After tumor tissue was dissected, total RNA was purified and subjected to real-time RT-PCR for the individual MT isoform mRNAs. This was compared to the level of glyceraldehyde-3-phosphate dehydrogenase mRNA in each sample. MT-1E, -1X and -2A were detected in both MT positive as well as MT negative tumors indicating that for these tumors the determining factor for the presence of MT protein was not the transcription of the MT gene. This is the first evidence in prostate cancer of post-transcriptional regulation of MT protein levels and is similar to the resent findings from this lab on normal breast tissue where both the myo- and ductal epithelial cells express ample amounts of mRNA for MT but only the myoepithelial cells express MT protein.

2040 STUDIES ON BLOOD METALLOTHIONEIN AS A BIOMARKER OF TISSUE METALLOTHIONEIN EXPRESSION

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Metallothionein (MT) is a multifunctional, low-molecular weight, metal-binding protein. MT reduces the toxicity of many metals and our recent work showed that poor expression of MT in mice predisposes them to the carcinogenic effects of several metals, including cadmium and lead. Tissue MT expression shows wide inter-individual variation in humans. However, surprisingly little is known about how blood MT levels might correlate with tissue expression. Thus, in order to help develop potential biomarkers to identify populations sensitive to metal toxicity and carcinogenesis, this study examined the correlation between blood MT and tissue MT expression in rodents. Male CD-1 mice and F344 rats were each divided into three groups (n = 4 to 5) including untreated controls, animals treated with zinc (100 µmol/kg, sc daily for 4 days), or animals treated with ethanol (50%, 5 ml/kg, ig daily for 7 days). One day after the last treatment, blood, liver, kidneys, and brain were collected. Total RNA was extracted, and real time RT-PCR was used to detect MT-1 and MT-2 transcripts in mice, or MT-1 transcript in rats. In liver and kidney of both mice and rats, MT transcript levels were dramatically increased by zinc, while ethanol only modestly increased levels. Statistical analysis in mice of pooled data from all groups showed a highly significant correlation between liver, kidney, and brain MT-1 and MT-2 mRNA levels and corresponding blood MT transcripts. Similar analysis in rats showed MT-1 transcript in liver, kidney and brain was also significantly correlated with blood MT-1 mRNA levels. In mice and rats MT protein levels, as assessed by ELISA or Cd-heme assay, in liver and kidney correlated with hepatic and renal MT RNA levels. In addition, blood MT transcript levels were significantly correlated with liver and kidney MT protein levels. This study indicates that blood MT transcript levels might be useful as an indicator of tissue MT protein and transcript levels, at least in some tissues.

2041 DOWN REGULATION OF MT-3 EXPRESSION IN HUMAN PROXIMAL TUBULE CELLS DECREASES DOME FORMATION AND ATTENUATES APOPTOSIS

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Epithelial cells possess a diverse number of functional properties and one of the functions of the proximal tubule epithelial cells *in vivo* is transepithelial solute transport. In cell culture, primary human proximal tubule cells retain this differentiated function of vectorial transport as evidenced by multi-cellular domes. Previous work from this laboratory has shown that the third isoform of metallothionein (MT-3) is expressed in the human kidney in situ, including the cells of the proximal tubules. Overexpression of MT-3 in a proximal tubule cell line HK-2 that lack its expression restores dome formation in these cells, suggesting that MT-3 may have a role in differentiation and vectorial active transport in the proximal tubule cells. Further studies provide evidence that MT-3 could play a role in controlling the choice between apoptosis and necrosis in response to cadmium exposure. The role of this study was to determine if down regulation of endogenous MT-3 in primary cultures of human proximal tubule cells had any effect on dome formation and the mode of cell death in response to cadmium treatment. For this purpose, small interfering RNA (siRNA) specific for MT-3 were transfected into human proximal tubule cells. Assessment of the cultures was done on the seventh day with counting of domes and harvesting of cells for MT-3 expression. Down regulation of endogenous MT-3 expression by siRNA significantly reduced the number of domes. The cultures with reduced number of domes were further treated with different doses of cadmium for various time periods and stained with 4',6-diamidino-2-phenylindole (DAPI) for visualization of fragmented nuclei. Treatment with cadmium resulted in an increase in the number of apoptotic nuclei. The knock down of MT-3 further implicates the role of MT-3 in dome formation and attenuation of apoptosis.

2042 OVER EXPRESSION OF METALLOTHIONEIN IN HUMAN BLADDER CANCER IS CORRELATED TO POOR PROGNOSIS

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The metallothioneins (MT) are a family of low molecular weight (6kD), intracellular proteins that have a very high conserved number of cysteine residues that allow the efficient binding of transition metals. These proteins serve an important role in the homeostasis of essential metals such as Zn^{+2} or Cu^{+2} during growth and development as well as in the detoxification of heavy metals such as Cd^{+2} and Hg^{+2}, rendering the MTs important mediators and attenuators of heavy metal-induced toxicity. It has been reported that the overexpression of the MT-1 and MT-2 isoforms can predict bladder cancers that will be resistant to treatment with cisplatin. The goal of the present study was to determine the extent of MT-1 and MT-2 overexpression in human bladder cancer. A total of 350 archival cases of formalin-fixed, paraffin-embedded bladder cancer specimens were examined for MT-1/2 immunoreactivity using the E-9 antibody. An examination of 137 specimens judged to be benign disclosed no immunoreactivity for MT-1/2. Of 13 specimens with dysplasia, 1 specimen was positive for MT-1/2. Of 59 cases diagnosed as low grade carcinoma, none were immunoreactive for MT-1/2. Two of 14 cases of *Carcinoma in-situ*(CIS) were found to stain for MT-1/2. Of 55 high grade cases that were non-invasive, 5 were positive for MT-1/2. In contrast, of 65 high grade cases that were invasive, 21 were immunoreactive for the MT-1/2 protein. In all cases of MT-1/2 positivity, staining was focal and localized to the cytoplasm of the cell. No tumor contained a majority of MT-1/2 immunoreactive cells. The presence of MT-1/2 immunoreactivity increased with tumor grade and correlated with a poor prognosis. These results suggest an association of MT-1 and MT-2 overexpression with the type and the grade of the tumor, with the more aggressive cancers having the highest level of MT-1/2 expression.

2043 BEHAVIOR OF BISMUTH ADMINISTERED INTRATRACHEALLY TO RATS

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Purpose: Bithmuth(Bi) which has similar physicochemical property to lead, has been expected to be used as lead substitute in solder, but its toxicity by inhalation is little known. In the present study, the distribution of Bi in rat organs and its time-depending changes were observed to clarify the biological behavior of Bi particles administered intratracheally.
Experiment: Male SD rats were administered once intratracheally with Bi particles suspended in 0.5% carboxymethylcellulose sodium solution at doses of 0, 20, 100, and 500 mg/kg body weight. Five rats in each group were dissected at 2,4,8, and 15 days after administration. Bi concentrations in lung, liver, kidney, and spleen were determined by high-frequency plasma mass-spectrometry after microwave digestion.
Results and discussion: Bi concentrations in lung increased with dose and decreased with days after administration. Distribution amounts of Bi in lung showed individual differences, and corresponded to 30-60% of dose at 2nd day and 20-25% at 15th day. Bi concentrations in kidney were higher than that in lung at 15th day and those in liver and spleen were very low. These results suggested that a part of Bi in lung remained for more than 2weeks and other part would be moved to blood flow and accumulated in kidney. Pathological changes in lung were observed in 100 and 500 mg/kg groups. Excretion behaviors of Bi in urine and feces are under investigation.

2044 UBIQUITINATION AND DEGRADATION OF EUKARYOTIC TRANSLATION INITIATION FACTOR 4E RESULT IN TOXICITY AND DEATH IN HELA CELLS EXPOSED TO POTASSIUM DICHROMATE

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Exposure of HeLa cells to 6.0 µM potassium dichromate (Cr) resulted in cytotoxicity, cell death and a significant reduction in the cellular level of eukaryotic translation initiation factor 4E – the rate-limiting factor required for mRNA translation.

Specific silencing of the eIF4E gene's expression with a small interfering RNA (siRNA) also resulted in significant cytotoxicity and cell death. Furthermore, the eIF4E silenced cells were significantly more susceptible to the Cr-induced cytotoxicity compared with the control cells suggesting that the Cr-induced toxicity in HeLa cells was, at least in part, due to the decreased cellular level of eIF4E protein. The Cr-induced reduction in the eIF4E protein level in HeLa cells was independent of the gene's transcription. Pre-exposure of the cells to ALLN and MG132 - inhibitors of proteasome activity, blocked the Cr-induced degradation of eIF4E protein. Inhibitors of PI3 kinase (LY294002), mTOR (rapamycin), and MAP kinase (SB203580, PD98059) blocked the Cr-induced degradation of eIF4E protein. Pretreatment of HeLa cells with insulin enhanced the phosphorylation as well as the Cr-induced degradation of the eIF4E protein. In addition, site-directed mutation at serine 209 - the phosphorylation site required for activation of the eIF4E protein, abolished its degradation induced by Cr in HeLa cells. These results confirmed that the phosphorylation of eIF4E protein is required for its ubiquitination and degradation in HeLa cells treated with Cr. The cellular level of cyclin D1 - a protein required for cell cycle progression was significantly lower in HeLa cells treated with chromium. Similarly, the siRNA-mediated silencing of the eIF4E gene's expression in HeLa cells also resulted in a significant reduction in the cellular level of cyclin D1 protein. In summary, our results demonstrate that the Cr-induced ubiquitination and degradation of the phosphorylated eIF4E protein in HeLa cells resulted in a decreased cellular level of cyclin D1 protein leading to cytotoxicity and cell death.

2045 ALPHA-TOCOPHEROL PREVENTS CHANGES ON THE CLAUDIN-2 AND OCCLUDIN EXPRESSION AND LOCALIZATION PATTERN, INDUCED BY POTASSIUM DICHROMATE IN MURINE KIDNEY

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Epithelial and endothelial tight junctions (TJs) are critical to maintain the permeability barrier required to preserve discrete compartments in the body. These proteins regulate the passage of ions, water and molecules through the paracellular pathway. Dichromate (CrVI) induces nephrotoxicity in humans, causing alterations in the traffic of diverse compounds that are transported through paracellular pathway along the nephron. Alpha-tocopherol (α-TOC) has protective effects on some the renal alterations caused by CrVI exposure. However, no information is available on the effect of this antioxidant on the paracellular pathway. We studied the time course of the localization and the expression patterns of Claudin-2 (Cl-2) and Occludin, proteins of the TJs, during the nephrotoxicity caused by CrVI, in the presence and in the absence of α-TOC treatment. Two experimental series were performed: 1) Wistar female rats received CrVI (15 mg/kg, SC, single dose, n=6); and 2) rats were pre-treated with α-TOC (125 mg/kg, orally, daily, n=6), initiating five days before and during the different periods of evaluation: control, 2 and 7 days after CrVI administration.
Cl-2 and Occludin were evaluated by: a) immunohistochemical techniques using frozen kidney slices, and b) Western blot of proximal tubules isolated by Percoll gradients. In control group microscopy immunofluorescence showed that both proteins are located at the cell borders of proximal tubular cells. In contrast, at day 2 after CrVI exposure, Cl-2 in the membrane was diminished accompanied by an increment in cytoplasmic location and on day 7 basal conditions were recovered. Alterations in the TJs proteins were prevented by pretreatment with α-TOC, indicating a protective role of this drug. (Supported by Conacyt-México grant G34511M, and grant ECOS-ANUIES MO2-SO2).

2046 CHROMIUM (VI) INDUCES ANTIOXIDANT GENE HO-1 BY ACTIVATING THE CNC BZIP TRANSCRIPTION FACTOR NRF2

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Chromium, a transition metal element abundantly present in the earth's crust, is widely used in industrial processes. Occupational exposure to Cr (VI)-containing compounds is known to cause multi-organ toxicity including renal damage, allergy and asthma, and cancer of the respiratory tract in humans. The molecular mechanism by which chromium elicits its biological effects is currently unclear but may involve the formation of reactive intermediates, oxidant stress, and gene induction. Here, we report that Cr (VI) induces antioxidant gene HO-1 at both mRNA and protein levels in hepa1c1c7 cells. Expression of a dominant negative form of Nrf2, a cap 'n' collar basic leucine zipper transcription factor, in the cells blocks the induction by Cr (VI), implicating Nrf2 as the key transcription factor in the induction. Mechanistic analysis reveals that Cr (VI) increases the protein but not the mRNA level of Nrf2 as well as the nuclear accumulation of the protein. Cr (VI) does not affect the protein levels of Keap1, which is a repressor of the cytoplasmic Nrf2, or Cul-3, an E3 ligase involved in proteasomal degradation of Nrf2. These results provide the first evidence that Cr (VI) induces antioxidant gene HO-1 important in ROS defense by activating Nrf2. The study offers a model in which transcriptional gene regulation by Cr (VI) can be analyzed at molecular levels.
The findings and conclusions in this abstract have not been formally disseminated by the National Institute for Occupational Safety and Health and should not be construed to represent any agency determination or policy

2047 CHROMIUM(VI) REQUIRES HISTONE DEACETYLASE TO INDUCE INTERFERON-STIMULATED GENES

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Cr(VI) promotes lung injury and is known to inhibit the inducibility of protective genes. A recent report indicated that this inhibition involves retention of histone deacetylase-1 (HDAC) activity and chromatin compaction in the proximal promoters of these genes (Wei et al. *J. Biol Chem* 279: 4110-4119, 2004). However, this mechanism would oppose any means for Cr(VI) to induce genes. To test the hypothesis that Cr(VI) can stimulate functional transcriptional complexes to induce genes, we screened for the binding of proteins to 156 different transcriptional DNA *cis-elements* in BEAS-2B in control and Cr(VI) exposed cells. Cr(VI) significantly increased the DNA binding of homodimers and heterodimers of multiple members of the signal transducer activator of transcription (STAT) protein family. Western analysis demonstrated that STAT1 was tyrosine phosphorylated and translocated to the nucleus within one hour of exposing the cells to 5 μM Cr(VI). Transient transfection with luciferase reporter constructs driven by STAT1 responsive interferon-stimulated response elements (ISRE) or gamma interferon activated sites (GAS) demonstrated that Cr(VI) stimulated functional STAT1 transactivation only at ISRE sites, suggesting the Cr(VI) stimulated a partial interferon (IFN) α/β-like response. In contrast to most genes, IFN-stimulated genes are induced by HDAC recruiting RNA polymerase II to functional transcription complexes containing ISRE elements. Rapid induction of the IFN-α stimulated gene IRF-7 by Cr(VI) was inhibited in cells previously incubated with the HDAC inhibitor sodium butyrate. These data present a novel mechanism for Cr(VI) to act through HDAC to induce genes, such as those stimulated by IFN involved in innate immune responses that are potentially cytostatic or cytotoxic. *Supported by NIEHS grant ES10638*.

2048 COMET ASSAY ANALYSIS OF DNA DAMAGE INDUCED BY CHROMIUM PICOLINATE

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Chromium picolinate (CrPic) is a popular dietary supplement, and as such it is not regulated by the FDA. CrPic has previously been shown to be clastogenic and mutagenic in cultured cells. The spectrum of mutations in the hprt locus of CHO AA8 cells was found to be significantly different from that reported to arise spontaneously, and consisted of base substitutions (38%) deletions (58%) and 1-4 base pair insertions (4%). The purpose of the current work was to identify the types of DNA lesions found in cells after exposure to CrPic to see if resulting DNA lesions were consistent with (i) the observed mutation spectrum, and (ii) with the proposal that CrPic may undergo Fenton-type chemistry to generated free radicals. The alkaline comet assay showed an increase in tail moment with increasing dose of CrPic, suggesting the presence of DNA strand breaks. Post-treatment exposure to methyl methanesulfonate (MMS) increased tail moment by 7-fold in untreated cells, but only increased tail moment from 5- to 2-fold in CrPic-treated cells, suggesting the presence of DNA crosslinks. Post-treatment exposure to formamidopyrimidine DNA glycosylase (FPG) had no affect on untreated cells, but increased the tail moment from 4- to 2-fold with increasing dose of CrPic, suggesting the presence of oxidative damage. The presence of oxidative damage was further supported by the observation that hprt mutations were decreased by more than 2-fold in cells exposed to CrPic dissolved in DMSO, a known radical scavenger, relative to cells exposed to CrPic in an acetone slurry. These data supported our working hypotheses that CrPic can cause direct DNA damage in cultured cells, and that mutations can arise from these lesions. Results suggest that further study is needed to verify the safety of CrPic for human consumption. Supported by NIH grant #CA75298, the Arizona Board of Regents Biotechnology and Human Welfare Program, and the NAU Minority Student Development Program (NIH #GM56931).

2049 LOSS OF REV3 PROTECTS AGAINST CR(VI) MUTAGENESIS IN S. CEREVISIAE

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The reduction of hexavalent chromium (Cr(VI))-containing compounds can lead to a variety of mutations and chromosomal abnormalities. Given that Cr leads to a variety of DNA lesions, it is likely that the mutagenic and clastogenic effects of Cr

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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, and poster sessions of the 45th Annual Meeting of the Society of Toxicology, held at the San Diego Convention Center, San Diego, March 5–9, 2006.

An alphabetical Author Index, cross referencing the corresponding abstract number(s), begins on page 500.

The issue also contains a Keyword Index (by subject or chemical) of all the presentations, beginning on page 534.

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

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