high substrate concentrations indicating that cytotoxic concentrations of NA do not stimulate the preferential release of proteins into the medium. Incubation of airways with ^{14}C-NOL resulted in higher levels of bound metabolites in medium than in tissue at 10, 50, 100 μM substrate. The pattern of labelling was similar to that with NA. Total RM bound from NN (250 μM) and from 4-IPO (5, 50 and 500 μM) were higher in tissue than in medium. These studies suggest that the pattern of RM binding in isolated airways is similar for both NA and NOL and that this pattern reflects the relative amounts of individual proteins present in tissue/medium. Supported by NIEHS 04311, 04699 and 06700.

803 ANTIBODY DETECTION OF COVALENT BINDING OF QUINONE METABOLITE OF NAPHTHALENE TO CLARA CELL PROTEINS

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Cytotoxicity of naphthalene is associated with the covalent binding of its reactive metabolites to proteins. 1,2-Naphthoquinone was found to covalently bind to cysteine residues of proteins of Clara cells incubated with naphthalene (Zheng et al *Toxicologist* 1994, 14, 115). To further identify the target proteins of the quinone metabolite of naphthalene, we raised polyclonal antibodies by immunizing rabbits with 1,2-naphthoquinone carrier protein adducts and used the antibodies as a probe to detect naphthoquinone cysteine moiety of the protein adducts. A high titer of polyclonal antibodies was obtained by antiserum dilution tests. Competitive ELISA showed that the antibodies specifically recognize 1,2-naphthoquinone *N*-acetyl cysteine adduct. No cross activity toward *N*-acetyl cysteine and its naphthalene oxide adduct was observed. For covalent binding studies, we incubated fleshly isolated Clara cell with naphthalene (0.5 mM). The cell homogenates were developed by SDS-PAGE, followed by Western blotting and immunostaining using the polyclonal antibodies we raised. Three protein bands with *Mr* 27, 28 and 52k dalton were detected by the antibodies. This finding indicates that the quinone derived metabolite of naphthalene selectively binds to Clara cell proteins *in vitro*.

804 TOXICITY OF N-ACETYL-p-BENZOQUINONE IMINE (NAPQI) AND DIQUAT IN CHINESE HAMSTER OVARY (CHO) CELLS

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Glutathione reductase (GR) protects tissues from oxidant injury by catalyzing the reduction of glutathione disulfide (GSSG) to glutathione (GSH). To study the role of GR under different oxidant stresses, we transfected CHO cells with an antisense cDNA for human GR by using a full length expression vector positioned antisense and downstream from the human metallothionein IIa promoter and co-transfected with a neomycin resistance gene to obtain a stable cell line. These cells (G17) have about half the GR activities of the parent CHO cells. Acetaminophen (AP), at concentrations up to 100 mM showed no toxicity in either cell line. NAPQI caused dose-dependent depletion of GSH in both CHO and G17 cells, from initial concentrations of 51.8 and 38.6 nmol/mg pro, respectively. Dose-dependent cell toxicity, as assessed by LDH leakage, also was observed, but with no difference in the two cell lines. As we have observed previously in studies on the effects of tert-butyl hydroperoxide in these cells, the GR-deficient G17 cells were more susceptible than were the parent CHO cells to the toxicity of diquat. Supported by NIH GM44263 and the Society for Pediatric Research.

805 INVESTIGATION OF HEPATOPROTECTION AFFORDED BY REPEATED EXPOSURE TO ACETAMINOPHEN

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Acetaminophen (A) toxicity is associated with its biotransformation to N-acetyl-p-benzoquinone imine, which, after glutathione is depleted, binds to protein thiols as 3-(cystein-S-yl)A-protein adduct (3-Cys-A). Previously, we reported remarkable resistance to supra-toxic doses of A (up to 65 g/day) in a physician who ingested progressively more A in codeine- and A-containing tablets as a consequence of Percocet addiction. To emulate this, mice were pretreated for eight days with increasing doses of A (A-pretreated). Controls received saline (S-pretreated). S-Pretreated mice challenged with 500 or 1000 mg/kg A on day 9 developed typical hepatotoxicity and the higher dose was lethal. In contrast, A-pretreated mice were protected as evidenced by an almost 400% increase in LD50. A-Pretreatment resulted in a >40% decrease in hepatic P450, decreased CYP2E1 activity, and >40% decreases in microsomal CYP2E1 and CYP1A2. Immunohistochemical staining for 3-Cys-A revealed an atypical distribution of A-protein adducts. Staining for PCNA revealed normal cell proliferative activity in S-pretreated animals, but increased proliferative activity as a consequence of A-pretreatment. Collectively, the data indicate that the association between covalent binding and toxicity may become uncoupled in A tolerance; that the mechanism of protection involves both inactivation of P450 and an ongoing cell proliferative response (autoprotection); and that the assessment of toxicity following repeated or chronic A exposure must consider parameters besides those used to assess acute A overdose.

806 IMMUNOCHEMICAL DETECTION OF PROTEIN ADDUCTS IN MICE TREATED WITH TRICHLOROETHYLENE

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Trichloroethylene is an industrial chemical with widespread occupational exposure and is a major environmental contaminant. It has been associated in the causation of the life threatening autoimmune disorder systemic sclerosis-like syndrome. The mechanism may involve the covalent modification of proteins that elicit an autoimmune response. To investigate whether formation of trichloroethylene-protein conjugates is involved in sclerosis-like syndrome, we synthesized an immunogen by derivitizing keyhole limpet hemocyanin (KLH) with dichloroacetic anhydride. Rabbits were immunized with dichloroacetic anhydride-KLH, and produced a high titer of antiserum that recognized dichloroacetic anhydride-treated rabbit serum albumin in an ELISA. Anti-dichloroacetic anhydride-KLH was used to detect modified proteins on Western blots of mouse liver fractions obtained 3 hours after dosing with 0, 500, or 1000 mg/kg of trichloroethylene. Several cytosolic protein adducts were detected, whereas the microsomal and 10,000xg pellet fractions did not contain detectable adducts. In a separate experiment, mice were pretreated for 8 days with 0.1% acetone to induce CYP 2E1 and then were injected *i.p.* with 0, 1000 or 2000 mg/kg of trichloroethylene. Western blots of liver and lung showed the presence of several protein adducts, the most prominent being 47–52 kDa in the microsomal fraction, suggesting binding to CYP 2E1. These results show that trichloroethylene binds to discrete protein fractions in mouse liver and lung; further analysis is required to elucidate the potential of these modified proteins to elicit autoimmunity.

807 MECHANISM OF ACETAMINOPHEN HEPATOTOXICITY: COVALENT BINDING VERSUS OXIDATIVE STRESS

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The hepatotoxicity of acetaminophen is mediated by the metabolite N-acetyl-p-benzoquinone imine. This species, which is both an electrophile and an oxidizing agent, may covalently bind to critical proteins as acetaminophen-protein adducts or may cause oxidative damage. The toxicity may be initiated by either mechanism. To determine which occurs first we administered a hepatotoxic dose of acetaminophen (400 mg/kg) to mice and assayed for acetaminophen-protein adducts at 0, 1, 2, 4, and 6 hours by a Western blot assay using an anti-acetaminophen antibody. We also assayed for increased protein aldehyde formation (a hydroxyl radical catalyzed reaction) by a Western blot assay which uses an initial derivitization with 2,4-dinitrophenylhydrazine followed by an anti-dinitrophenyl antibody. Acetaminophen increased serum ALT levels at 4 and 6 hours, but not at 1 or 2 hours. Acetaminophen plus $FeSO_4$ (100 mg/kg) significantly increased serum ALT at 2 hours and levels were higher than acetaminophen alone at 4 and 6 hours. Fe alone did not increase ALT; however, Fe alone increased the intensity of staining in the Western blot assay for protein aldehydes over control at 2, 4, and 6 hours. Acetaminophen did not cause an increase in protein aldehydes over control at any time, nor did acetaminophen plus Fe. Acetaminophen-protein adducts were detected in acetaminophen and acetaminophen plus Fe mice at 2 and 4 hours. Acetaminophen appears to quench protein oxidation and presumably the oxidative process. These data are consistent with covalent binding as the primary mechanism of toxicity and argue against a role for oxidative stress in acetaminophen hepatotoxicity.

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