Western blot. This process was also significantly inhibited by zinc. The results obtained from this study thus demonstrates that zinc specifically inhibits caspase-3 activation, suggesting its anti-apoptotic effect is mediated by multiple actions on the mitochondrion-controled apoptotic pathway. (Supported in part by NIH grants CA68125 and HL59225.)

530 DOXORUBICIN-INDUCED HEPATOTOXICITY MAY INVOLVE APOPTOTIC CELL DEATH BY MODULATING EXPRESSION OF BCL-XL AND P53.

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The cytotoxic effect of Doxorubicin (DOX) on malignant cells and its toxic effects on various target organs such as liver, heart, and kidney are well known. This study examined the potential of DOX to induce or enhance apoptotic cell death, and to influence the expression of apoptosis-regulatory genes such as bcl-XL and p53 in the liver. Male ICR mice, fed ad libitum, were treated ip with single doses of DOX (20, 40, 60, 80 and 120 mg/kg) and sacrificed 36 hours later. Blood was collected for analysis of serum chemistry, and liver samples for histopathology and Western blot analysis. DOX produced liver injury (>ALT levels) and DNA fragmentation in a dose-dependent manner. Higher doses of DOX (80 and 120 mg/kg) were lethal to mice. Lower doses (0, 20, 40, 60 mg/kg) inhibited bcl-XL expression in a dose-dependent manner (nearly blocking it at 40 and 60 mg/kg), and increased apoptotic death, whereas the same doses of DOX enhanced p53 gene expression. Although p53 is known to induce either cell cycle arrest or apoptosis, these findings suggest that p53 facilitates apoptotic death. (Supported by Div. of Pharmacol. & Toxicol., AMS Coll. of Pharm. & Hlth. Scs.)

531 OXIDATIVE STRESS IN KERATINOCYTES: ROLE IN APOPTOTIC SIGNALING

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We studied the mechanism(s) through which oxidative stress participates in apoptotic signaling in keratinocytes. We exposed normal human keratinocytes (NHEKs) to cumene hydroperoxide (Cu-OOH, 50-200μM) for 1 h and found significant and dose-dependent depletion of antioxidant reserves, oxidation of GSH and protein sulfhydryls. We metabolically labeled membrane phospholipids in NHEKs with oxidation-sensitive cis-parinaric acid (PnA) to detect selective oxidation of specific phospholipid classes. We found that incubation of NHEKs with Cu-OOH resulted in dose-dependent oxidation of PnA-phospholipids: phosphatidylinositol (PI), phosphatidylserine (PS), phosphatidylethanolamine (PE) and phosphatidylcholine (PC). Most importantly, PS was significantly more sensitive to Cu-OOH oxidation than other phospholipids. This selective oxidation of PS did not occur in liposomes prepared from PnA-labeled keratinocytes phospholipids. Exposure to Cu-OOH induced externalization of PS in NHEKs, as evidenced by its chemical labeling with fluorescamine. These oxidative modifications of NHEK phospholipids were not accompanied by gross changes of the phospholipid composition as evidenced by our HPTLC determinations. Together with other assays of apoptosis (caspase-3 activation and DNA laddering), our results suggest that Cu-OOH-induced selective PS oxidation may represent a signaling pathway linked to PS externalization in keratinocytes undergoing apoptosis.

532 ENHANCED GLUTATHIONE BIOSYNTHESIS RETARDS APOPTOSIS IN SPITE OF CASPASE-3 ACTIVATION IN HEPA-1 CELLS OVEREXPRESSING GLUTAMATE-CYSTEINE LIGASE.

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Glutathione is a major antioxidant that is responsible for maintaining thiol redox potential. Glutamate-Cysteine Ligase (GLCL) is the rate limiting enzyme in glutathione biosynthesis. Increased glutathione has been implicated in the resistance of certain cell types to apoptotic stimuli. We have stably transfected mouse liver Hepa-1 cells (CR17) which overexpress both the catalytic (GLCLc) and regulatory (GLCLr) subunits of GLCL, to test the hypothesis that increased glutathione synthesis is important in preventing apoptosis. Previously we have shown that CR17 cells when treated with TNF plus actinomycin D conserve their glutathione levels, NADPH levels and mitochondrial membrane potential, whereas plasmid vector alone transfectants (Hepa-V cells) did not. Caspase-3 is activated in both Hepa-V and CR17 cells with TNF+ActD treatment at 12 hrs. Western blot analysis shows substantial cleavage of GLCLc in Hepa-V cells, whereas only minimal cleavage of GLCLc was seen in CR17 cells at 22 hr. The increased glutathione biosynthesis capacity of CR17 cells not only allows them to resist TNF+ActD induced glutathione depletion, but may also inhibit apoptotic events secondary to caspase-3 activation. (This work was supported by NIH Grants ES04696, ES07033 and ES07032.)

533 COMPARATIVE *IN VITRO* STUDIES OF CADMIUM AND ARSENIC-INDUCED APOPTOSIS IN RENAL TUBULE EPITHELIAL CELLS.

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Cadmium and arsenic are nephrotoxic elements which frequently occur together in Superfund sites. The mechanism(s) by which these elements produce cell death are not completely understood. The present studies were undertaken to examine differences in the inter-species induction of apoptosis in renal tubule epithelial cells from rats, hamsters and humans following *in vitro* exposures to Cd^{2+} or As^{3+} over concentration ranges between 10-7M to 10-4M. The TUNEL assay was utilized to examine the induction of apoptosis in relation to cell injury as monitored by changes in ALAMAR Blue fluorescence. Results of these studies indicated that both elements were capable of producing apoptosis in cells from all 3 species but that the cellular mechanisms appear different for Cd^{2+} and As^{3+} since there were marked morphological response differences for each element. Arsenic (As^{3+}) exposure also produced necrosis in some cells so that both necrosis and apoptosis mechanisms were operating. The results of the ALAMAR Blue assay showed a dose-related decreases in cell metabolism following both cadmium and arsenic exposure. Overall the results of these studies indicate that renal tubule epithelial cells from all 3 species are susceptible in a similar manner to apoptosis induced by Cd^{2+} and As^{3+} exposure. It is hypothesized that the As^{3+} is operating via mitochondrial apoptotic inducing factors while the mechanism for cadmium is presently unclear. (Supported by USEPA STAR Grant ER 827161-01-0.)

534 ACROLEIN ENHANCES MECHLORETHAMINE-INDUCED APOPTOSIS.

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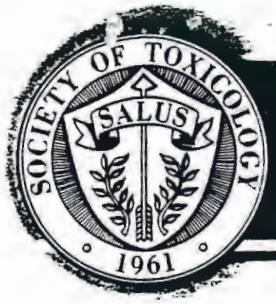
Acrolein, a highly electrophilic α,β-unsaturated aldehyde is, along with phosphoramide mustard, a metabolic product of cyclophosphamide (CP). Although acrolein is generally considered to be a toxic metabolite, at low concentrations it may enhance the activity of CP by sensitizing cells to apoptosis. To test this theory, B lymphocytic FL5.12 cells were incubated for 0.5 h with 2.5 μM acrolein followed by 150 or 250 nM mechlorethamine. Acrolein depleted GSH by ~80% at 0.5 h. Apoptosis was morphologically distinguished from necrosis by the acridine orange/ethidium bromide assay. At 24 h post-treatment, acrolein alone caused 15% apoptosis and 6% necrosis, whereas mechlorethamine alone caused 23 and 33% apoptosis and 5 and 2% necrosis at 150 and 250 nM, respectively. When acrolein and 150 nM mechlorethamine were combined there was 44% apoptosis and 4% necrosis. Acrolein with 250 nM mechlorethamine caused 60% apoptosis and 5% necrosis. Enhanced apoptosis was more evident using an ELISA to assess nucleosomes in the cytoplasm. To test the possibility this sensitization effect was working through GSH depletion, FL5.12 cells were treated with 1 mM diethyl maleate (DEM) (lowering GSH by ~70%). Surprisingly, DEM alone caused a significant amount of necrosis, making it impossible to assess the effect of co-treatment with mechlorethamine. These data demonstrate that acrolein enhances apoptosis induced by subsequent treatment with an alklyating agent. At comparable levels of GSH depletion, the effects of acrolein and DEM differ suggesting mechanistic differences. The ability of acrolein to inhibit NF-κB activation at 4 h may be a factor in the observed apoptosis. (Supported by HL48035, ES09791 and ES07784.)



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