formation of PARs, partially attenuated TGHQ-mediated ATP depletion, but had no effect on NAD depletion. Intriguingly, although z-vad-fmk (a pan-caspase inhibitor) attenuated TGHQ-induced apoptosis, co-treatment with PJ-34 led to a further decrease in apoptosis. The findings suggest that PARP participates in caspase activation during the apoptotic response to DNA-damage. Indeed, PARP-1 inhibition appears to reduce TGHQ-induced capase-3,-7, and -9 activation by attenuating cytochrome C translocation from mitochondria to the cytoplasm. In contrast, PJ-34 potentiated TGHQ-induced caspase-8 activation, suggesting that PARP-1 plays a dual role in regulating TGHQ-induced apoptosis via opposing effects on the intrinsic (mitochondrial) and extrinsic (death-receptor) pathways. Finally, TGHQ-induced cell death was accompanied by the nuclear accumulation of apoptosis-inducing factor (AIF), and PJ-34 inhibited TGHQ-induced AIF nuclear translocation. Neither JNK nor p38 MAPK activation were required for AIF translocation, since PJ-34 actually enhanced p-JNK and p-p38-MAPK levels. In summary, TGHQ-induced apoptotic cell death of HL-60 cells is accompanied by PARP-1 and caspase activation, and AIF nuclear translocation. TGHQ-induced apoptosis appears to primarily occur via engagement of the mitochondrial-mediated pathway in a process amenable to PARP inhibition. The residual cell death in the presence of PJ-34 is likely mediated via the extrinsic apoptotic pathway. (Supported by the SWEHSC [P30 ES06994-16].

PS 2045 PYRROLIDINE DITHIOCARBAMATE AUGMENTS HG2+ IN INDUCING MACROPHAGE CELL DEATH THROUGH OXIDATIVE STRESS-INDUCED APOPTOSIS AND NECROSIS SIGNALING PATHWAYS.

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Exposure to mercury causes several types of injuries in mammals, including immune system dysfunction. In addition, environmental factors may enhance the cytotoxic effects of mercury. Thus, it is important to understand and explore the possible toxic mechanisms of mercury combined with environmental factors. Here, we demonstrated that pyrrolidine dithiocarbamate(PDTC), a commonly used antioxidant and metal chelator, augmented HgCl2-induced cytotoxic effects by facilitating mercury entry into the cultured murine macrophage (RAW 264.7 cells). The Hg2+/PDTC complex significantly and rapidly increased ROS formation and decreased intracellular GSH levels in cells. By means of a flow cytometric technique, the number of sub-G1 hypodiploids and Annexin V-FITC binding cells were found to be increased after Hg2+/PDTC complex exposure. Several features of mitochondrial-dependent apoptosis were also induced, including mitochondrial dysfuntion, activations of poly (ADP-ribose) polymerase (PARP) and caspase 3/7, and DNA fragmentation. Moreover, both apoptotic and necrotic cells were detected by acridine orange/ethidium bromide dual staining. Meanwhile, depletion of intracellular ATP levels and increased LDH release were observed, suggesting the induction of necrotic cell death processes. All of these Hg2+/PDTC complex-induced cytotoxic-related signals could be reversed by pre-treated with an antioxidant, N-acetylcysteine. In conclusion, the results obtained suggest that Hg2+/PDTC complex-induced oxidative stress caused macrophage cell death via a mixed type of apoptosis and necrosis. These findings imply for first that PDTC may enhance the uptake of Hg2+ and dramatically enhance the toxicological effects of Hg2+ instead of detoxification.

PS 2046 A COMBINATION OF ANTIAPOPTOTIC/ANTINECROPTOTIC INHIBITORS PROTECTS NCCIT CELLS FROM GAMMA-IRRADIATION-INDUCED CELL DEATH.

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Irradiation-induced cell death includes apoptotic and necroptotic (or programmed necrosis) pathways. Therefore, a search for radiomitigators with the delayed action against acute radiation injury due to radionuclide release in terrorist acts may be based on a combination of antiapoptotic and antinecroptotic inhibitors. Here we used a cocktail that included a pan-capase inhibitor, Z-VAD-fmk, and necrostatin-1, a specific inhibitor of receptor interacting protein-1 (RIP-1) - a key protein in necroptosis - and assessed their protective effects against irradiation-induced (4 Gy) death of embryonic carcinoma NCCIT cells (5 days after irradiation). We found that a combination of Z-VAD-fmk (50-100 microM) plus necrostatin-1 (20 microM) showed significant radiomitigative effect – from 33.5%-61.9% in NCCIT cells (treatment at 30 min after the irradiation exposure). No significant protection was afforded by either of these compounds alone. Our results suggest that necroptosis inhibitors – along with other therapeutic modalities - may be promising as mitigators against late radiation-induced cell death. Supported by NIOSH OH008282; NIH U19 AI068021, HL70755, HL094488, ES020693, ES021068.

PS 2047 CYTOTOXICITY MEDIATED BY EGFR INHIBITION IS OVERCOME BY NOX-INDUCED AUTOPHAGY IN HEAD AND NECK CANCER CELLS.

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Epidermal Growth Factor Receptor (EGFR) is overexpressed in most head and neck squamous cell carcinomas (HNSCC) which makes it an attractive candidate for molecular targeted therapies. Treatment regimens involving EGFR inhibitors and radiation has demonstrated clinical effectiveness, however, many HNSCC tumors become resistant to EGFR inhibitors. Previous work in our lab has shown that the EGFR inhibitor Erlotinib, induces oxidative stress and cancer cell killing via NADPH Oxidase 4 (NOX4). Autophagy (the process involving the breakdown of cellular components with the help of lysosomal machinery) is activated by oxidative stress and has recently been reported to confer resistance to chemotherapy. The purpose of this study is to determine if the EGFR inhibitor Erlotinib induces autophagy in FaDu and Cal-27 HNSCC cells via NOX4. Erlotinib-induced cytotoxicity (as determined by clonogenic assay) in FaDu and Cal-27 HNSCC cells compared to control treated cells. Erlotinib-induced the expression of autophagy marker LC3B-II in both cell lines as determined by western blot and immunofluorescence assays. Treatment with DPI (diphenyleneiodonium) in the presence of Erlotinib inhibited the increase in LC3B-II expression induced by Erlotinib in FaDu and Cal-27 cells. Finally, knockdown of NOX4 using adenoviral siNOX4, partially suppressed the activation of LC3B-II in FaDu and Cal-27 cells. These results show that Erlotinib activates autophagy in HNSCC cells, and NOX4 may play a role in mediating this effect. Furthermore NOX4-induced autophagy may play a role in conferring resistance to EGFR inhibitors. Supported by KO1CA134941

PS 2048 PREDICTING PRECLINICAL OUTCOMES BY MEASUREMENT OF LYSOSOMAL DYSFUNCTION.

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The effective prediction of *in vitro* tools during preclinical drug development is essential when applying them toward early safety assessment. We developed an *in vitro* screen to bin compounds according to lysosomal mass based on fluorescent intensity and area, allowing project teams the ability to explore chemical trends. During validation of this assay with a group of over 40 marketed drugs, numerous data trends were observed comparing lysosomal changes to physical chemical properties and overall *in vivo* rat LD50 values. Briefly, HepG2 cells were plated in 96 well plates and treated with compound for 24 hours. Following treatment, cells were washed and stained with 50 nM lysotracker green DND-26 and 10μg/mL Hoechst for one hour, followed by data acquisition on the Acumen Explorer. Analyses of these data against known *in vivo* outcomes showed that lysosomal accumulation *in vitro* correlated inversely with *in vivo* rat LD50 values. Surprisingly, neither lysosomal mass nor *in vivo* LD50 values trended with published volume of distribution data (L/kg) in the rat. However, there were physical chemical trends that correlated to an increase in HepG2 lysotracker staining, including high lipophilicity (clogP > 2.5) and basicity (pKa > 6), most of which also exhibited low LD50 values *in vivo*. Compounds which did not cause a change in lysotracker staining from control had higher observed *in vivo* LD50 values and were noted as having lower lipophilicity (clogP < 2.5) and/or basicity (pKa < 6). While direct measurement of lysosomal accumulation appears to be a critical link to *in vivo* outcomes, several outliers appeared in the data set, such as wortmanin, which exhibited negative staining for lysotracker and was non-cytolethal but had a low LD50 (4 mg/kg) *in vivo*. Interestingly, not all compounds that increased lysotracker accumulation caused phospholipidosis. Taken together, these data imply that incorporating additional functional endpoints relevant to lysosomal accumulation and normal vesicular trafficking could play an important role in predicting adverse preclinical outcomes *in vivo*.

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