PS 2054 CHRONIC EXPOSURE TO CADMIUM INDUCES APOPTOSIS THROUGH THE ACCUMULATION OF P53 IN KIDNEY OF MICE.

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We have found that overaccumulation of p53 by cadmium (Cd) may relate to induction of apoptosis and may be due to the suppression of p53 degradation through the inhibition of expression of ubiquitin-conjugating enzyme genes, Ube2d family in rat proximal tubule cells (NRK-52E cells). In this study, we examined the effect of chronic exposure to Cd on the expression of Ube2d family genes and accumulation of p53 in the mouse kidney. Five weeks old female C57BL/6J mice were fed diet containing 300 ppm Cd without restraint for 12 months. Cd slightly elevated blood urea nitrogen value but not creatinine value in serum. Some of mice exposed Cd was detected histopathological change (e.g., urinary casts and cell swelling) in the renal tubules. Thus, Cd-induced renal toxicity was weakly developed. Ube2d family (Ube2d1, Ube2d2 and Ube2d3) mRNA levels significantly decreased and p53 accumulated in the kidney of Cd group. Moreover, apoptotic cells were predominantly detected in renal tubules and were absent in glomeruli of all Cd-exposed mice. These results suggest, therefore, that Cd causes p53-dependent apoptosis in not only NRK-52E cells but also the renal tubules of mice, and that Cd-induced accumulation of p53 may be due to down-regulation of Ube2d family genes in the kidney of mice.

PS 2055 APPLICATION OF *IN VITRO* CYTOLETHALITY AND PROLIFERATION ASSAYS TO IMPROVE KINASE INHIBITOR SELECTIVITY PROFILE AND REDUCE PRECLINICAL HEMATOTOXICITY.

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Hematotoxicity, leading to myelosuppression and neutropenia, is the most common clinical dose-limiting toxicity (DLT) encountered during development targeted kinase inhibitor compounds. Hematotoxicity may be driven by multiple biological, chemical and physicochemical (pChem) properties. In these studies, rat primary hepatocytes were utilized to assess pChem toxicity, and bone marrow mononuclear cells were employed to evaluate biologic (on- and off-target) activity. Rat primary hepatocytes were isolated and assessed for cytolethality after treatment with different concentrations of compounds for 24 hours. Bone marrow mononuclear cells, KG-1 meyloblasts, or CD34+ bone marrow cells were incubated with different concentrations of compound for either short term (4 – 24 hrs) or long-term exposure (10 – 14 days). Inhibition of proliferation (IC50, IC90) or cytolethality (LC50) values were calculated and used with pChem and kinase selectivity data to categorize compound risk. Greater viability was observed with compounds exhibiting better overall pChem properties and, therefore, was used as a prefilter to select compounds ahead of the proliferation assays. Kinase inhibitor compounds with improved selectivity, specifically against inhibition of CDK9, exhibited less inhibition of proliferation in the models utilized to assess hematotoxicity risk. In summary, we have applied a strategy that utilizes multiple *in vitro* tools to guide kinase inhibitor compounds toward optimal pChem properties and kinase selectivity, resulting in overall lower toxicity. Furthermore, the *in vitro* assay output translated to reduced hematotoxicity and increased margin of safety to dose-limiting myelosuppression prior to clinical development. This strategy has also produced a higher throughput surrogate assay to assess hematotoxicity risk that can be applied during preclinical drug development.

PS 2056 ROLE OF OXYGENATED PHOSPHATIDYLSERINE AND ITS METABOLITES—LYSO-PHOSPHATIDYLSERINES PRODUCED AFTER OXIDATION AND HYDROLYSIS BY PLASMA LIPOPROTEIN-ASSOCIATED PHOSPHOLIPASE A2 IN CLEARANCE OF APOPTOTIC CELLS BY MACROPHAGES: LC-ESI-MS STUDY.

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Peroxidized phospholipids and their metabolites are known modulators of inflammatory responses. We suggested that during apoptosis, oxidative modification of externalized on the cell surface phosphatidylserine (PS) and its subsequent hydrolysis by LpPLA2VIIA would yield a pattern of diversified molecular products with different affinity and recognition by macrophage (PS) receptors. To make HL60 cells susceptible to oxidation we treated them with linoleic acid (LA) (100 nmol/106 cells). We found that phagocytosis of LA-enriched apoptotic HL60 cells (100 μM H2O2) by RAW 264.7 macrophages was significantly higher compared to naïve apoptotic HL60 cells and was suppressed by annexin V. Using oxidative lipidomics approach we were able to detect the presence of oxidized PS species containing LA with two and three oxygens in LA-enriched apoptotic HL60 cells. Further, we determined whether treatment of LA-enriched apoptotic HL60 cells with LpPLA2VIIA will affect their phagocytosis. We found that the ability of macrophages to engulf apoptotic cells was significantly reduced after treatment of cells with LpPLA2VIIA. In addition, MS analysis revealed the presence of lyso-PS and oxygenated LA accompanied by a decrease amounts of oxidized PS (but not non-oxidized PS). Further, we oxidized C18:0/C18:2-PS (cyt c/H2O2) and integrated it into naïve HL-60 cells. We found that phagocytosis of HL60 cells containing oxygenated PS on cell surface was ~two time higher compared to cells with incorporated non-oxidized PS. We suggest that oxidatively modified externalized PS and its hydrolysis products are important represents regulators of phagocytosis and inflammatory responses. Supported by a contract with GlaxoSmithKline, OH008282, U19AI068021, HL70755, HL094488, ES020693

PS 2057 INTRACELLULAR ACCUMULATION OF SULFIDES AND INDUCTION OF APOPTOSIS DEPEND ON PH IN NAHS-EXPOSED JURKAT CELLS.

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Hydrogen sulfide (H_2S) is a toxic gaseous substance and has an odor of rotten eggs. Accidental exposure to high concentrations of H_2S has been reported to be lethal in human. Inhaled and absorbed H_2S may be partially ionized in blood and cause toxic effects on lymphocytes (pK_1 of H_2S is 6.8 at 37 °C). However, the mechanism for toxicity of H_2S has not been well documented. In this study we studied cellular uptake and cytotoxicity of sulfides in human lymphoma cells (Jurkat) following in vitro exposure to NaHS at different pHs. The cells were exposed to 0-5 mM NaHS in HBSS in a screw-capped plastic conical tube for 1 hr at 37 °C with gentle shaking. The pH of HBSS was adjusted to pH 6.0, 7.0 or 8.0 and the air was removed by filling the tube with HBSS to avoid a loss of dissolved H_2S gas from HBSS. The cells were collected by centrifugation and cultured in RPMI1640 culture medium for 6-24 hrs in a culture dish. The cytotoxicity of NaHS increased with the decrease of pH in HBSS. The cell viability was not changed by the pH in the absence of NaHS. The activity of caspase-3/7 in NaHS-exposed cells was measured by colorimetric method and was found to be increased with the decrease of pH in HBSS. Western blotting using anti-PARP and anti-caspase-3 also revealed that exposure to 5 mM NaHS at pH 6.0 induced apoptosis. Z-VAD-fmk, a pan-caspase inhibitor, reduced the NaHS-induced activation of caspase-3, indicating that pH-dependent cell death caused by NaHS was due to activation of caspases. The concentration of sulfides in the cells was measured by an HPLC with a fluorescent detector. The cellular sulfide concentration in NaHS-exposed cells (5 mM) increased dramatically as pH decreased. At pH 6.0 the proportion of H_2S form, which is permeable through the cell membrane, to less permeable HS^- and S^{2-} forms is higher compared that at pH 7.0-8.0. Therefore the larger number of cells underwent apoptosis in the acidic condition.

PS 2058 EFFECT OF SURFACE-MODIFIED TITANIUM BY CHEMICAL TREATMENT ON THE GENE EXPRESSION PROFILE IN OSTEOGENIC DIFFERENTIATION OF HUMAN MESENCHYMAL STEM CELLS.

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Titanium is the material widely used for orthopedic and dental applications. Surface properties of material play a major role in cell-material interaction. Adult human mesenchymal stem cells (hMSCs) have the pluripotency to differentiate into cells of mesodermal origin, e.g., bone, cartilage, adipose, and muscle cells. In the present study, we evaluated the molecular responses of hMSCs to three modified titanium surfaces. Experimental titanium disks were treated with NaOH, NaOH+$CaCl_2$, and NaOH+$Ca(OH)_2$. Untreated titanium disks served as control. Then, hMSCs were cultured on each surface-modified titanium disk for 7 days. Comparative gene expression profile changes in hMSCs were assessed by the DNA microarray. The gene expressions in hMSCs cultured on three kinds of chemically treated surfaces were compared with that on untreated titanium. The expressions of

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