cells. The present experiments have been carried out in order to investigate whether this effect can be prevented by VR1 receptor antagonists. The capsaicin uptake was assessed by HPLC separation and quantification on C18 reverse-phase column and fluorescence detection using, respectively, 270 nm and 330 nm as excitation and emission wavelengths. The cytotoxicity was assessed by incorporation of [3H]L-leucine into cellular proteins in the presence of capsazepine, the competitive vanilloid receptor antagonist, Ruthenium red, tyrosine and calcium. Capsazepine (1-200 μM) did not modify the uptake rate of capsaicin and did not antagonise CPS-induced protein synthesis inhibition. Ruthenium red inhibited protein synthesis and did not interfere with capsaicin effects. Interestingly, calcium chloride (10-50 μM) antagonised the cytotoxic effects of capsaicin in a DMEM medium deprived of calcium. Tyrosine inhibited CPS cytotoxicity, preventing its metabolism but not its diffusion. The results of the present study suggest that cell responses to capsaicin may be transduced through multiple pathways, only some of which involve VR1. Capsaicin might enter cells by diffusion, interfere with protein synthesis machinery by competition with tyrosine which, in turn, prevents the metabolism and binding of capsaicin or its metabolites to cellular macromolecules.

192 POSSIBLE INVOLVEMENT OF MAPK PHOSPHORYLATION OF NRF2 IN THE REGULATION OF GCS_l GENE EXPRESSION *VIA* AN ELECTROPHILE RESPONSE ELEMENT.

L. M. Zipper and R. T. Mulcahy. *University of Wisconsin, Pharmacology, Madison, WI.* Sponsor: A. Elfarra.

One of the transcription factors mediating up-regulation of genes containing Electrophile Response Elements (EpREs, also called Antioxidant Response Elements, AREs) is Nrf2. The light subunit of γGlutamylcysteine Synthetase, the rate limiting enzyme in glutathione synthesis, is up-regulated by Nrf2 binding to the EpRE within the GCS_l promoter. Although Nrf2/GCS_l EpRE binding increases after exposure to pro-oxidants, Nrf2 mRNA levels remain constant, suggesting that post-translational events are involved in Nrf2 binding the GCS_l EpRE. Inhibition of the MAP Kinases ERK and p38 inhibits GCS_l up-regulation and Nrf2 binding to the GCS_l EpRE, suggesting the possibility that ERK and/or p38 might phosphorylate Nrf2 to affect EpRE binding and activation. Consistent with this possibility, cross-species comparison of Nrf2 amino acid sequences, revealed several conserved MAPK consensus phosphorylation sites. By use of a GST-Nrf2 fusion protein, we have demonstrated that Nrf2 is an *in vitro* substrate for ERK. Preliminary data obtained using 2-dimensional seperation of protein extracts followed by western analysis with a Nrf2 antibody to detect changes in pI, suggest that Nrf2 phosphorylation may occur in vivo following exposure to the pro-oxidant PDTC. Mutation of individual consensus MAPK phosphorylation sites decreases basal Nrf2 activation of EpRE gene expression. Similar analysis will evaluate the role of phosphorylation at these sites during induction of EpRE transcription. This work was supported by NIEHS09749 and T32-CA09471.

193 SELECTIVE OXIDATION OF THE N-TERMINAL AMINE OF APO B-100 BY MYELOPEROXIDASE (MPO) *IN VITRO.*

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In contrast to the twelve 2,4-dinitrophenylhydrazine (DNPH)-reactive peptides we isolated and characterized from low-density lopoproteins (LDL) oxidized with reagent hypochlorous acid (HOCl), MPO-mediated oxidation of LDL *in vitro* produced a single major tryptic peptide that showed DNPH-dependent absorbance at 365 nm. This peptide exhibited the gas-phase N-terminal sequence, VELEVPQL(*C)SFILK., corresponding to amino acid residues 53 through 66 in apo B-100. Subsequent mass spectral analyses with MALDI-ESI QqTOF and additional mass spectral data indicate that the N-terminal amino group of apo B-100 is oxidized selectively by MPO (in the presence of Cl- and H_2O_2) to the respective ketone or other DNPH-reactive precursor, possibly through the chloroamine with dehydrohalogenation to the imine, followed by hydrolysis. The N-terminal sequence observed in sequence analysis is from the tryptic peptide 53-66 bound to the modified tryptic peptide by a disulfide linkage of the respective cysteine residues. The distinct differences between the products of oxidation observed in tryptic peptides from LDL oxidized *in vitro* with reagent HOCl and the modified peptides obtained with MPO-catalyzed oxidation clearly contradict the hypothesis that MPO-catalyzed oxidation of LDL is mediated by free HOCl. The selectivity of the reaction mediated by MPO suggests a direct interaction between the LDL particle and the active site on MPO, resulting in the surprising selectivity of product formation. The results indicate how strongly the protein microenvironment can affect product formation and stability. Supported by 97G-213 and 0050530 from the AHA and GM 44263 from the NIH.

194 ZN PROTECTS METALLOTHIONEIN WILDTYPE (MT+/+) BUT NOT METALLOTHIONEIN NULL (MT-/-) LUNG FIBROBLASTS AGAINST CU-NTA-INDUCED APOPTOSIS.

V. E. Kagan, S. X. Liu, J. F. Jiang, C. M. St. Croix, J. P. Fabisiak and B. R. Pitt. *University of Pittsburgh, Environmental and Occupational Health, Pittsburgh, PA.*

Pretreatment of HL-60 cells with Zn prompts their resistance to oxidative stress and apoptosis induced by copper nitrilotriacetate (Cu-NTA) likely due to induction of metallothioneins (MTs) and their ability to sequester redox-active Cu. Protective effects of Zn, however, may be due to induction or repression of different genes including but not limited to MTs. In order to specifically assign the effects of Zn-pretreatment to MT induction we compared the effects of Zn in lung fibroblasts derived from either MT1,MT2 knockout mice (MT-/-) mice or wildtype (MT+/+) mice. In the absence of Zn, MT+/+ cells contained approximately 0.10 μg MT/mg protein while MT null cells expressed 10-fold less MT (as evidenced by Cd109 binding assay). ZnCl2-pretreatment (100 μM, 48 h) resulted in over a ten-fold induction of MT in MT+/+ cells whereas MT-/- cells still contained MT at levels 100-fold less than their MT wildtype counterparts. MT-/- cells were highly sensitive to Cu-NTA-induced apoptosis both in the presence and in the absence of ZnCl2-pretreatment. In contrast, ZnCl2-pretreated MT+/+ cells exerted only very low sensitivity to Cu-NTA. In the absence of ZnCl2-pretreatment, MT+/+ cells demonstrated moderate resistance at low Cu-NTA concentrations but were as sensitive as MT-/- cells at high Cu-NTA concentrations. We conclude that ZnCl2-induction of MTs and their ability to sequester intracellular Cu is solely responsible for the observed Zn-dependent protection against Cu redox-cycling activity and subsequent apoptosis. Supported by NIH HL64145-01A1, HL32154-17 and EPA STAR R827151.

195 LIPOATE/ASCORBATE-DEPENDENT RECYCLING PREVENTS MYELOPEROXIDASE-CATALYZED OXIDATION OF VITAMIN E HOMOLOGUES' PHENOXYL RADICAL IN LIVE HL-60 CELLS.

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Effectiveness of the antioxidant system in cells is believed to be due to antioxidant recycling mechanisms. Several antioxidant cascades including ascorbate/thiols and enzymatic electron carriers have been suggested to participate in vitamin E recycling based on experiments in cell-free systems. We report that thiol-driven redox-cycling of vitamin E operates in live HL-60 cells. Myeloperoxidase-rich HL-60 cells were able to generate, in the presence of H_2O_2, phenoxyl radicals of 2,2,5,7,8-pentamethyl-6-hydroxy chromane (PMC), a vitamin E homologue devoid of the hydrocarbon side-chain. The steady-state concentrations of PMC phenoxyl radicals were high enough to be directly detectable by EPR spectroscopy. Pretreatment of HL-60 cells with succinyl acetone, an inhibitor of heme synthesis, resulted in a decreased activity of myeloperoxidase and an alleviated levels of PMC phenoxyl radicals. Preloading with ascorbate (achieved by the addition of dehydroascorbate to the growth medium for 1 h) resulted in disappearance of PMC phenoxyl radical signal and appearance of ascorbate radical signal in the cells exposed to PMC. HPLC measurements confirmed that myeloperoxidase-catalyzed PMC consumption was delayed and oxidation of ascorbate was detected during this period. Pretreatment of the cells with lipoate and dehydroascorbate further postponed PMC oxidation and increased the effectiveness of ascorbate in recycling PMC phenoxyl radical as evidenced by both EPR measurements and HPLC assays. We conclude that electron transfer reactions in the recycling cascade (dihydro)lipoate->ascorbate->PMC phenoxyl radical was operational in live HL-60 cells.

196 A CRITICAL EXAMINATION OF THE CAUSE-AND-EFFECT RELATIONSHIP BETWEEN METALLOTHIONEIN INDUCTION AND ITS PROTECTION FROM OXIDATIVE INJURY.

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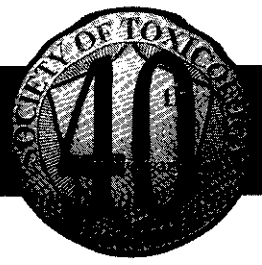
Many studies have shown that metallothionein (MT), a potent antioxidant, was increased to a significant high level under a diversity of oxidative insults in multiple organ systems. Yet, the increase in MT production often failed to show a protective



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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, roundtable, and poster sessions of the 40th Annual Meeting of the Society of Toxicology, held at the Moscone Convention Center, San Francisco, California, March 25–29, 2001.

An alphabetical Author Index, cross referencing the corresponding abstract number(s), begins on page 451.

The issue also contains a Keyword Index (by subject or chemical) of all the presentations, beginning on page 479.

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