789 ANTIOXIDANT LOADING PROTECTS FROM BLAST OVERPRESSURE-INDUCED OXIDATIVE STRESS.

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Detonation of explosives or firing of large caliber weapons during military operations or training and occupational high energy impulse noise, produce blast overpressure (BOP) waves characterized by rapid rise in atmospheric pressure above ambient. Exposure to BOP waves can cause injury, predominantly to the hollow organs accompanied by hemorrhage, edema, and hypoxia. We observed in rats that BOP induces free radical-mediated oxidative stress characterized by antioxidant depletion, increased lipid peroxidation, and decreased hemoglobin (Hb) oxygenation. We examined whether loading the rats with pharmacological doses of antioxidants can protect from BOP. Sprague-Dawley rats weighing 300-350 g were loaded with vitamin E (vE), vitamin C (vC) or lipoic acid (LA) for 3 consecutive days before a BOP exposure. Antioxidants were administered by gavage using 2-ml vehicle (corn oil or distilled water) alone or containing 800 IU vE, 25 mg LA or 1000 mg vC. Each regimen was subdivided into 4 groups (6 rats/group): vehicle control and exposed, and antioxidant control and exposed. After the 3-day loading, all rats were deeply anesthetized with sodium pentobarbital, then the exposed groups were subjected to a low-level BOP at 62±2 kPa peak pressure of 5 msec duration. One hour after exposure, the rats were euthanized then blood and lungs were analyzed for biochemical alterations. We found that antioxidant loading resulted in elevated Hb oxygenation, decreased lipid peroxidation, and smaller lung weight increases. These observations suggest that preloading with pharmacological doses of antioxidants for only three days can alleviate BOP-induced oxidative stress. The order of antioxidant efficacy was vE>LA>vC. These observations have clinical and occupational implications.

790 FREE RADICAL SCAVENGING *VS* BCL-2 INDUCTION IN ANTIAPOPTOTIC EFFECT OF ESTROGENS IN MCF-7 BREAST CANCER CELLS.

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Since estrogens are known to act as radical scavengers their antiapoptotic activity may be related the antioxidant effects. Alternatively, this activity may be due to estrogen receptor- and estradiol-induced overexpression of the anti-apoptotic gene, *bcl-2*. To experimentally resolve these alternative pathways we studied long-term and acute effects of estrogens on phospholipid peroxidation induced in MCF-7 human breast cancer cells by a lipid-soluble azo initiator of peroxyl radicals 2,2'-azobis(2,4-dimethylvaleronitrile), AMVN (addition of 10^{-8}-5×10^{-7}M estradiol to the medium immediately or for 14 days preceding AMVN treatment). Incubation of control MCF-7 cells with AMVN resulted in oxidation of major phospholipid classes: phosphatidylinositol (PI), phosphatidylserine (PS), phosphatidylethanolamine (PE) and phosphatidylcholine (PC). Acute exposure of MCF-7 cells to estradiol resulted in protection against AMVN-induced oxidation of PI and PS, and a trend towards such protection of PE and PC. Long-term exposure to estradiol caused selective protection of PS, whose oxidation is a critical component of the final common pathway for apoptosis. Not surprisingly, protection of PS was accompanied by a 2-fold decrease in the percentage of cells demonstrating apoptotic morphology after a 24 h exposure to AMVN. Additionally, long-term exposure to estradiol yielded an increase in GSH level in MCF-7 cells while short-term incubation had no effect of GSH content. Our results indicate that estrogens may act as both free radical scavengers and antiapoptotic agents in breast cancer cells.

791 MYELOPEROXIDASE-CATALYZED ONE-ELECTRON GENERATION OF ETOPOSIDE PHENOXYL RADICALS IN VIABLE HL60 CELLS.

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Etoposide (VP-16), a widely used phenolic antitumor drug, is also known to cause secondary acute myeloid leukemias. We hypothesized that VP-16 genotoxicity is associated with its one-electron oxidative metabolism to its phenoxyl radical catalyzed by myeloperoxidase (MPO) in bone marrow progenitor cells. Our previous work demonstrated that both purified MPO and MPO activity in homogenates of human leukemia HL60 cells were able to catalyze formation of VP-16 phenoxyl radicals in the presence of H_2O_2. In this study, we attempted to identify conditions compatible with the detection of VP-16 phenoxyl radicals in viable HL60 cells. We found that VP-16 phenoxyl radical could be directly observed in HL60 cells by EPR spectroscopy if MPO activity was sufficiently high (≥ 10 nmol quaiacol/min/10^6 cells), endogenous GSH was substantially depleted (> 75% by 5 min preincubation with a maleimide SH reagent, ThioGlo™-1), and (*iii*) endogenous catalase was inhibited (by pretreatment with 3-amino-1,2,4-triazole). In HL60 cells grown in the presence of an inhibitor of heme synthesis, succinyl acetone (≤ 500 μM), both decreased MPO activity and VP-16 phenoxyl radical production was observed. Importantly, a significantly enhanced VP-16-induced formation of DNA/topoisomerase II complexes was found in HL60 cells under conditions where GSH concentration was depleted by 54% as a result of ThiolGlo™-1 treatment (1.0 μM, 5 min) and VP-16 phenoxyl radical was immediately detectable by EPR. We conclude that VP-16 phenoxyl radical formation may be essential for VP-16-induced geno- and cytotoxicity through either their direct effects on topoisomerase II and/or indirectly on intracellular GSH.

792 REDOX-CYCLING OF PHENOLS CAUSES DEPLETION OF GSH, OXIDATIVE STRESS AND CYTOTOXICITY IN NORMAL HUMAN EPIDERMAL KERATINOCYTES (NHEKS)

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Exposure to phenolic compounds is associated with neuro-, myelo-, immuno-, geno-, and dermotoxicity. In skin, phenols cause rash and inflammation, contact and irritant dermatitis, leucoderma and cancer promotion. The biochemical mechanisms responsible for cytotoxicity of phenolic compounds are not well understood. We hypothesized that the cytotoxic effects of phenolic compounds are due, at least in part, to the generation of phenoxyl radicals via the enzymatic one-electron oxidation which alters the intracellular pool of GSH and protein sulfhydryls. To test this hypothesis, we measured cell viability, intracellular levels of GSH and protein SH-groups, as well as lipid peroxidation in NHEKs exposed to twelve different phenolic compounds. We found that an 18 h incubation of cultured NHEKs in the presence of 100-500 μM 1,4-benzenediol (hydroquinone), *o*-hydroxybiphenyl (2-phenylphenol), bis (4-glycidyloxyphenyl)-methane, bis (4-hydroxyphenyl)-dimethylmethane (bisphenol A), 4-*tert*-butylcatechol, 1,2-benzenediol (catechol), *iso*-eugenol (2-methoxy-4-propenylphenol), eugenol (4-allyl-2-methoxyphenol) or phenol caused pronounced cytotoxicity and significant depletion of GSH. In addition, concentration-dependent cell detachment, loss of cell-cell contacts, and disruption of the cellular membrane were observed. Importantly, the phenolic compounds that did not decrease thiol levels, such as 3-n-pentadecylphenol, 4-*tert*-butylphenol, or bis (4-hydroxyphenyl) methane, did not induce cytotoxicity after a 18 h treatment. Incubation of ascorbate-preloaded keratinocytes with phenols produced an EPR-detectable signal of ascorbate radicals indicating that redox-cycling of the one-electron oxidation product of phenol, its phenoxyl radical, is likely involved in these oxidative effects. While phenolics are known to act as radical scavengers, their enzymatically formed metabolites, phenoxyl radicals, can react with vital thiol reductants and cause cytotoxic effects.

793 OXIDATIVE STRESS FOLLOWING TRAUMATIC BRAIN INJURY IN RATS: ASCORBATE RADICAL AS A TOOL TO DETECT FREE RADICAL ACTIVITY.

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Several studies failed to detect generation of oxygen radicals following traumatic brain injury (TBI) using ESR of spin traps. We attempted to detect any spin adducts of oxygen radicals in the ESR spectra of brain samples taken at different times after TBI (1.0, 2.5, and 6 hr) from animals injected with high doses of DMPO (ip, 80 mmol/kg). We found that the concentration of DMPO in the brain 1 h after injection was ≈10.0 mmol/kg and declined by 6h to ≈2.0 mmol/kg. We demonstrated that endogenous ascorbate was able to outcompete DMPO as the radical scavenger, hence prevent any detection of DMPO adducts with oxygen radicals. We used a catalytic system to generate hydroxyl radicals (H_2O_2/Fe^{2+}) in brain homogenates in which we manipulated concentrations of DMPO and endogenous ascorbate (using ascorbate oxidase).

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