The Pro-Oxidant Chromium(VI) Inhibits Multiple Core Mitochondrial Functions: EPR Markers for Multiple Iron-Sulfur Proteins

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Chromium [Cr(VI)] compounds are strong oxidants that readily enter cells where they are reduced to reactive Cr intermediates that facilitate reactive oxygen species (ROS) generation. Previous findings showed that it can cause oxidation of mitochondrial thioredoxin (Trx2) and peroxiredoxin (Prx3) in human bronchial epithelial cells, implying that there is significant Cr(VI)-induced mitochondrial oxidant stress. Here we show that that Cr(VI) also causes pronounced and irreversible inhibition of aconitase, a TCA cycle enzyme that has an oxidant-sensitive iron-sulfur (Fe-S) center. Electron transport complexes I and II were also inhibited, whereas complex III was not. EPR studies of samples at liquid helium temperature (10 K) showed a strong signal at g = 1.94 that is consistent with the inhibition of electron flow through complexes I and/or II. A signal at g = 2.02 was also observed and was consistent with oxidation of the Fe-S center of mitochondrial aconitase. The g = 1.94 signal was particularly intense and remained after extracellular Cr(VI) was removed, suggesting irreversible disruptions in electron flow through complexes I and/or II. While the g = 2.02 signal declined in intensity after Cr(VI) was removed, aconitase activity did not recover suggesting irreversible disassembly of its Fe-S cluster. A similar inhibition of aconitase, complex I, and complex II, as well as analogous EPR findings were noted in bovine airways treated ex vivo with Cr(VI). This supports the biological relevance of the human cell culture studies. The g = 1.94 signal in particular could prove to be an important biomarker for mitochondrial oxidative damage resulting from Cr(VI) exposure. The EPR spectra simultaneously showed signals for Cr(V) and Cr(III) which verify Cr(VI) exposure and its intracellular reductive activation. Together with the previous findings, Cr(VI) exposure has deleterious effects on a number of redox-sensitive core mitochondrial functions that are essential for energy generation and the maintenance of mitochondrial redox status. These changes could have important implications for Cr(VI) cytotoxicity and cell survival.

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253

Synthesis and Characterization of new Anthracene Derivatives used as Singlet Molecular Oxygen Chemical Traps

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Anthracene compounds have been used like fluorescent probes because of their highly fluorescent nature. We synthesized some anionic and non-ionic anthracene derivatives with modification in the polycyclic aromatic ring, the synthesis is based on structural modifications in position 9-10 of dibromoanthracene, grafting hydrophilic substituents. Diethyl 3,3-(9,10-anthracenediylic) bisacrylate (DADB) and anthracene-9,10-divinylsulfonate (AVS) were obtained by Heck reaction of 1,9-dibromomethane with ethyl acrylate and aqueous sodium vinylsulfonate, respectively. DADB can be hydrolyzed generating the carboxylic acid, the diethyl 3,3-(9,10-anthracenediylic)diacrylate (DADD), by deprotonation reaction. These compounds can react with singlet molecular oxygen \(O_2^+(\lambda_{\alpha})\), a potential damage source in biological systems, yielding the corresponding endoperoxides. These compounds are characterized by two absorption bands centered around 405 and 260 nm and fluorescence emission around 525 nm with \(\lambda_{\alpha}=405 \text{ nm}\). However, the fluorescence can be affected by absence of the ethyl group in the carboxylic acid ester (\(\phi=0.08-0.30\)). Products analysis by LC/MS, UV/Vis and fluorescence of DADD, DADB, and AVS after reaction with a clean chemical source of \(O_2^+(\lambda_{\alpha})\) (1,4-dimethylnaphthalene endoperoxide) and photosensitization with methylene blue, showed the corresponding 9,10-endoperoxide generation. The bimolecular quenching constants (k) of these compounds ranged from 1-10 \(10^7 \text{ M}^{-1} \text{s}^{-1}\). Eletrochemical potentials were obtained and correlated with quenching constants, the lower the

DNA Damage and Cytotoxic Effects of the Ci Db291 Purified Dinitrophenylazo Dye in Hepg2 Cells

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CI Disperse Blue 291 (CI DB291) is used in the textile industry together with other dinitrophenylazo dyes. It is mutagenic in the Ames assay, but there are few studies showing its effects in eukaryotic cells. We evaluated here the toxicity of CI DB 291 in the human HepG2 cell line. CI DB291 dinitrophenylazo dye was purified through HPLC-PDA and used for HepG2 cell incubations (5, 10, 50, 100 µM of the dye for 24h). Cytotoxicity was assessed by the XTT, crystal violet dye (CVD), extracellular lactate dehydrogenase (LDH) and glucose consumption assays. The formation of ROS was measured by intracellular 2',7'-dichlorofluorescein (DCF) fluorescence. Cell cycle and DNA fragmentation analyses were done by flow cytometry. Oxidative damage was assessed through 8-oxodGuo, 1,N'-cAdAdo and 1,N'-cAdGuo HPLC-ESI-MS/MS analysis in DNA from cells incubated with 100 µM of the dye. The purified dinitrophenylazo dye significantly decreased cell survival (CVD) in a dose-dependent manner (IC50=74 µM). The XTT assay revealed an induction of formazan formation with 10 or 50 µM of dye, probably due to an increase in mitochondria enzyme activity. Glucose consumption rate increased in cells incubated with 50 µM of the dye. Cell death did not occur with plasma membrane lysis (LDH), indicating that the pathway is not necrosis. DCF fluorescence level increased after 24h incubation with 50 and 100 µM of dye, indicating the formation of ROS. DNA fragmentation and G2/M cell cycle arrest were also observed in a dose-dependent mode. The HPLC-ESI-MS/MS analysis of tree DNA lesions indicative of cell oxidative damage (8-oxodGuo, 1,N'-cAdAdo and 1,N'-cAdGuo) confirmed the induction of oxidative stress by the dye. The results show that the CI DB291 dinitrophenylazo dye may lead to cell toxicity and mutagenicity through the induction of oxidative stress.

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252
Can Ionizing Radiation Provide Resistance to Cyanide?  
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Peas are legumes widely consumed in the world and ionizing radiation (IR) is a non-conventional technique that reduces post-harvest losses. However, studies suggest that IR stimulates radical oxygen species (ROS) production and can affect respiration of plants leading to growth decreasing. This study aims to evaluate the effect of different doses of radiation on the respiratory capacity of pea seedlings during 7 days of germination. Consumption of O₂ was evaluated by Oxygraph Hansatech in irradiated seeds with 50 and 250 Gy during 7 days of germination. The radicle and caulicle growth until the 4th day remains in a similar profile in all groups of seeds, although after the 4th day growth inhibition were observed, in a dose-dependent way, of 49.01% and 68.1% in radicle and caulicle of irradiated seeds with 250 Gy, respectively. On the 5th day of germination, irradiated seeds showed less O₂ consumption compared to non-irradiated seeds in about 92.5% in radicle of 50 Gy seeds, and 75.8% in caulicle of 250 Gy. Interestingly, an addition of 10mM of glucose decreases O₂ consumption about 61% in caulicle in the 7th day of germination, 1mg/ml olygomicin does not promote inhibition of O₂ consumption in all doses tested, 1mM FCCP were unable to acelerate O₂ consumption in radicle of irradiated seeds on the 5th day of germination and 1mM cyanide does not inhibit O₂ consumption in irradiated seeds, but changed about 60% in non-irradiated ones on day 0. In conclusion, ionizing radiation inhibits the growth of seeds, reduces O₂ consumption, and provided resistance to cyanide, suggesting that ionizing radiation can affect respiratory capacity, probably by uncoupling the respiration to ATP synthesis.

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Antioxidant Enzyme Activities in Pacific White Shrimp (Litopenaeus Vannamei) in Response to White Spot Virus Infection  
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White spot virus (WSSV) is considered an extremely virulent pathogen that affects most of the crustaceans in both hemispheres. WSSV causes oxidative stress due to the production of ROS as part of the immune system that defends the host against foreign micro-organisms. Our study examined the antioxidant enzymes and oxidative damage associated with oxidative stress in tissues (haemolymph, digestive gland, gills and muscle) of Pacific white shrimp (Litopenaeus vannamei) infected by intramuscular injection of a viral inoculum. Shrimp were held in aquaria at 27°C, and samples were taken at different time intervals post-infection (0, 1, 24 and 48 h), viral load were confirmed and quantified by real time PCR. The levels of oxidative damage (lipid peroxidation and carbonylated proteins), the activities of antioxidant enzymes (superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase and glutathione S-transferase) were quantified in healthy and WSSV-infected shrimp tissues. All tissues showed marked differences at the course of infection. Lipid peroxidation and protein carbonyl levels were higher in WSSV-infected tissues compared to uninfected controls. A significant reduction in antioxidant enzymes activities was found at 48 h post-infection in all the tissues analyzed. These results could indicate oxidative stress and tissue damage via inactivation of antioxidant enzymes in WSSV-infected shrimps resulting in system failure and sudden death.

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Severe Liver Pathology Caused by Dietary Omega-6 PUFAs in Alcoholic Liver Disease may be Associated with COX-2 Mediated Lipid Peroxidation and TNF-α Mediated Pathways  
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Alcoholic Liver disease (ALD) has been associated with increased oxidative stress and free-radical mediated tissue damage. Increased free radical production in ALD has been correlated with degree of lipid peroxidation of polyunsaturated fatty acids (PUFAs) such as ω-3/ω-6 PUFAs. However, there is conflicting evidence regarding which PUFAs (ω-3 or ω-6) lead to development of more severe liver injury when administered with ethanol. Also, the mechanistic relationship by which these PUFAs...