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EFFECT OF ULTRAVIOLET B INDUCED OXIDATIVE STRESS IN CONJUGATION WITH COPPER ON HUMAN MELANOCYTES AND MELANOSOMAL PROPERTIES

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Melanin's most assumed function is protection against harmful effects of ultraviolet radiation (UVR). However, both exposure to high flux of energy and metal binding alter melanin properties. Herein we study the effect of ultraviolet B (UVB) on cultured human melanocytes from skin type I/II and compare its effect to melanocytes that were exposed to both Cu(II) and UVB. Our studies show that while exposure of melanocytes to low concentrations of Cu(II) alone doesn't seem to affect melanocytes, when combined with ultraviolet radiation, it amplifies the harmful effects of UVB in several different ways. Copper markedly increases the effect of UVB radiation on morphology of melanocytes including increasing dendricity and enlargement of the cell body. These morphological changes are associated with G2 cell cycle arrest, which is an indication of DNA damage. Ultrastructural studies of melanosomes in melanocytes exposed to UVB demonstrates that copper accelerates the bleaching of melanin, which reduces the photoprotective effect of the pigment upon further exposure to ultraviolet light. By stabilizing the oxidized form of melanin, as demonstrated by increased auto-fluorescence of melanosomes, copper binding decreases the buffering capacity of the pigment. Upon oxidation of copper-bound melanosomes genotoxic hydroxyl radicals are formed. Furthermore upon excessive oxidation of the pigment, copper will participates in Fenton reaction with UV generated hydrogen peroxide, leading to formation of hydroxyl radicals in high efficiency. Our results indicates possible contribution of copper to UVB induced melanocyte transformation and carcinogenesis through reducing photoprotection capacity of melanin and production of hydroxyl radicals.

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POSSIBLE ROLE OF CATALASE ACTIVITY AS A BIOMARKER FOR GLEEVEC-RESISTANT CML PATIENTS

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Introduction: Chronic Myeloid Leukemia (CML) is a hematologic malignancy cytogenetically characterized by a translocation on chromossomes 9:22. It leads to an overactivity of the enzyme tyrosine-kinase (TK), which by itself is responsible for the severity of the disease. The most efficient treatment for CML consists on inhibition of the TK by a especific biochemical inhibitor, named Gleevec® (Novartis). It's known that Abl TK phosphorilates the enzyme catalase, enhancing its activity. Catalase is an enzyme that regulates intracelular hydrogen peroxide (H_2O_2), producing O_2 and H_2O. Compaired to normal leucocytes, catalase 's activity is enhanced in CML.

Objective: The aim of this work is to find out wether the inhibition of Bcr-Abl TK is followed by an inhibition of catalase.

Methods: Supernatant of cell lysates from CML patients were treated overnight with Gleevec® 3μM and enzyme activity of catalase was measured by monitoring decomposition of 10mM H_2O_2 at 240nm according to the method described by Aebi (*Methods Enzymol*. 1984; 105:121-126).

Results: Specific activity of catalase was decreased in 50% of samples previously treated with Gleevec® in 3/8 patients. In contrast, catalase activity in samples from patients with clinical Gleevec® resistance (5/8) remained unchanged after incubation *in vitro* with Gleevec®.

Conclusion: Our preliminary results suggest that catalase activity might be a powerful biomarker for resistance to Gleevec® in CML patients.

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CHEMOSENSITIZATION OF PROSTATE CANCER CELLS BY DEOXYGLUCOSE.

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Prostate cancer cells are more metabolically active than their normal counterpart, but often hypoxic. They use anaerobic glycolysis to maintain the high proliferation rate and glucose is their primary and most important source of energy. Tumor cells respond to the increase in cellular glucose demand by upregulating the expression of the facilitative glucose transporters in their membrane. Therefore, tumor cells accumulate more glucose than normal cells and glucose deprivation induces a stress response that results in cytotoxicity and apoptosis of the tumor. Glucose deprivation chemosensitize cancer cells, however, the mechanism for sensitization is not well understood. Here we targeted the glucose metabolism of prostate cancer cells with the non-metabolized glucose analog 2-deoxy-D-glucose (DOG) that induces glucose deprivation by competing for transport and intracellular accumulation of glucose. Prostate cancer cells treated with DOG showed a time and dose dependent decrease in cell viability, that was associated with the generation of reactive oxygen species (ROS). Antioxidants inhibited both cell death and ROS production. We found that the mechanism of DOG-induced cell death correlates with induction of mitochondrial membrane depolarization. Importantly, DOG treatment induces sensitization of tumor cells, and the chemotherapeutics dose required to induce cell death was significant reduced in cells treated with DOG. Additional in vivo studies will explore the feasibility of using DOG in a clinical setting.

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THE IRON CHELATING CARDIOPROTECTIVE PRODRUG DEXRAZOXANE DOES NOT AFFECT THE CELL GROWTH INHIBITORY EFFECTS OF BLEOMYCIN

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The clinical use of bleomycin is limited by a dose-dependent pulmonary toxicity. Bleomycin is thought to be growth inhibitory by virtue of its ability to oxidatively damage DNA through its complex with iron. Our previous preclinical studies showed that bleomycin-induced pulmonary toxicity can be reduced by pretreatment with the doxorubicin cardioprotective agent dexrazoxane. Dexrazoxane is thought to protect against iron-based oxygen radical damage through the iron chelating ability of its hydrolyzed metabolite ADR-925, an analog of ethylenediaminetetraacetic acid (EDTA). ADR-925 quickly and effectively displaced either ferrous or ferric iron from its complex with bleomycin. This result suggests that dexrazoxane may have the potential to antagonize the iron-dependent growth inhibitory effects of bleomycin. A study was undertaken to determine if dexrazoxane could antagonize bleomycin-mediated cytotoxicity using a CHO-derived cell line (DZR) that was highly resistant to dexrazoxane through a threonine-48 to isoleucine mutation in topoisomerase IIα. Dexrazoxane is also a cell growth inhibitor that acts through its ability to inhibit the catalytic activity of topoisomerase II. Thus, the DZR cell line allowed us to examine the cell growth inhibitory effects of bleomycin in the presence of dexrazoxane without the