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SUPPRESSION OF NADH-DEHYDROGENASE ACTIVITY AND PRODUCTION OF SEMIQUINONE FREE RADICALS AS WELL AS ROS PRODUCTION IN HUMAN ERYTHROLEUKEMIA K562 CELLS UPON DEVELOPMENT OF RESISTANCE TO DOXORUBICIN

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Introduction: Mitochondrial NADH-degydrogenase promotes one-electron transfer with rise of semiquinones that are autooxidized with the formation of superoxide radical. We studied the state of ROS production and free radicals level under development of K562 cells resistance to doxorubicin (DOX). Methods: Detection of semiquinone free radicals level by ESR, ROS production by fluorescence method, antioxidant enzymes and NADH degydrogenase activities were tested in sensitive and resistant to DOX human erythroleukemia K562 cells. Results: Compared to sensitive cells significant decrease of NADH degydrogenase activity was found in K562/DOX cells. Accompanied to suppression of NADH degydrogenase activity the suppression of semiquinone free radicals production was detected in resistant cells. In addition development of resistance to DOX led to decrease of ROS production (superoxide anion radicals as well H_2O_2) in contrast to growth of total Cu,Zn-SOD and Mn-SOD activity which concluded more rapid increase of Mn-SOD activity. Increase of H_2O_2-catabolizing enzymes activities – catalase and glutathione peroxidase was added to the enhancement of antioxidant state in resistance cells. Conclusions: Development of resistance to DOX in K562 cells is connected with growth of adaptive antioxidant response.

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MITOCHONDRIAL ROLE IN ETOPOSIDE-INDUCED APOPTOSIS OF HUMAN PROSTATIC CANCER CELLS

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Androgen independent (PC-3 and Du 145) and dependent (LNCaP) human prostatic cancer cells in culture were exposed to etoposide for 12, 24 and 48 h. Apoptosis was characterized by morphological analysis with light and fluorescent microscopy, and by scanning and transmission electron microscopy. In the initial phase of apoptotis cell shrank with loss of cytoplasmic content and volume, became detached from their neighbor cells and from culture substrata and adapted a smooth contour. In the following phase, the plasma membrane showed ruffles and blebs. In the third phase, progressive degeneration of residual nuclear and cytoplasmic structures was observed. X-ray microanalysis showed a progressive decrease of intracellular K^+, with the corresponding increased intracellular Na^+. Mitochondria were isolated and respiratory rates and respiratory control were determined. Mitochondrial membranes show considerable biochemical damage, with increased levels of protein carbonyls and TBARS, as markers of damage by oxidative stress; with decreased mitochondrial electron transfer activities, and increased nitric oxide production. Dysfunctional mitochondria, with altered ultrastructure, increased products of free radical-dependent oxidation, and decreased respiratory function appeared in the apoptotic cells before the loss of plasma membrane selective permeability and nuclear DNA fragmentation.

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SELF-SENSITIZED PHOTODEGRADATION OF MEMBRANE PROTOPORPHYRIN IX (PpIX) MEDIATED BY FREE RADICAL LIPID PEROXIDATION: PROTECTION BY NITRIC OXIDE WITH PROLONGATION OF SINGLET OXYGEN ($^1O_2$) PRODUCTION

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Amphiphilic PpIX is a potent $^1O_2$-generating photosensitizer which mediates 5-aminolevulinate (ALA)-based photodynamic therapy (PDT). In addition to sensitizing lipid peroxidation and other damage during visible light exposure, membrane-bound PpIX itself slowly degrades. Using the cholesterol hydroperoxides 5a-OOH and 7a/b-OOH as $^1O_2$ and free radical reporters, respectively, we showed previously that diazeniumdiolate (e.g. 0.4 mM SPER/NO)-derived NO had no effect on the initial rate of 5a-OOH accumulation during irradiation of PpIX-containing liposomes (LUVs) in the presence of iron and ascorbate, but strongly inhibited 7a/b-OOH buildup from secondary chain peroxidation. We now report that 5a-OOH accumulation slows progressively with prolonged irradiation and that NO dramatically reverses this while suppressing 7a/b-OOH and free radical peroxidation. The extent of chain peroxidation and 5a-OOH slowdown correlated with degree of LUV unsaturation, decreasing from eggPC/Ch to POPC/Ch to DMPC/Ch LUVs. The 5a-OOH slowdown was accompanied by a decrease in $^1O_2$ quantum yield and PpIX fluorescence at 630 nm. We postulated that NO, by protecting PpIX, should prolong purely $^1O_2$-mediated damage. To test this, we used lactate dehydrogenase (LDH) as a non-membrane $^1O_2$ target. Irradiation of PpIX-containing LUVs in the presence of