

S10.17 Evaluation of antioxidant properties of some pyrazolo[3,4-d]pyrimidines derivatives and their effects on mitochondria bioenergetics

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In recent years, pyrazolopyrimidines and related fused heterocycles have been identified as bioactive molecules. They are known to function as CNS (Central Nervous System) depressants, neuroleptic agents, and as tuberculostatic. Pyrazolo[3,4-d]pyrimidines were identified as a general class of adenosine receptors. The aim of this work was to determine the antioxidant properties of some novel pyrazolo[3,4-d]pyrimidine derivatives once oxidative stress is thought to play an important role in numerous degenerative or chronic diseases, such as atherosclerosis and cancer. Our results show that some of these compounds have good antioxidant when using ABTS and DPPH methods. Furthermore, membrane protection against *tert*-butylhydroperoxide oxidation was observed by the decrease in the TBARS produced when mitochondrial membranes were pretreated with those compounds. We also evaluate their effect on mitochondrial bioenergetics. State 4, state 3 respiration, and mitochondrial membrane potential will be studied in the presence of the more antioxidant efficient compounds.

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S10.18 Interaction of mitochondria-targeted antioxidants with cells in culture

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Ubiquinone or plastoquinone conjugated via decane linker with triphenyl phosphonium cation (MitoQ and SkQ1, respectively) are shown to be powerful antioxidants in isolated mitochondria. We found that plastoquinone conjugated with positively charged fluorophore rhodamine-19 (SkQR1) selectively accumulates in mitochondria of HeLa cells and human fibroblasts, reaching plateau at 2 h. Uncoupler FCCP suppresses the accumulation and stimulates the release of SkQR1. Incubation with nanomolar SkQ1 or SkQR1 for 2 h prevents oxidation of glutathione and fragmentation of mitochondria induced by H₂O₂. However, 2 h incubation does not result in resistance of HeLa cells and human fibroblasts to H₂O₂ or other prooxidants (menadione, paraquat). The protective effect becomes pronounced only after 24 h incubation and disappears during 48 h after removal of quinones in parallel with SkQR1 release from the cells. Prolonged (6–7 d) incubation with 1–20 nM SkQ1 causes strong resistance against oxidative stress, MitoQ being 100 times less effective. Simultaneously, the antioxidants promote structural and functional fusion of mitochondria increasing the size of electrically-united mitochondrial network. Staining of mitochondria with SkQR1 for 2 h revealed their significant heterogeneity while after prolonged incubation mitochondria become uniformly stained. It is suggested that slow development of the antiapoptotic effect is related to slow

redistribution of mitochondria-targeted antioxidants between mitochondria in the cell.

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S10.19 Pro-oxidant and anti-oxidant properties of mitochondrial matrix-targeted ubiquinone MITOQ₁₀

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Addition of mitochondria-targeted coenzyme Q, MitoQ₁₀, to HEP-G2 cells with rotenone-inhibited mitochondrial Complex I sharply decreases rotenone-induced superoxide release to the matrix (J_m) and increases cell respiration. Thenoyltrifluoroacetone, a Complex II inhibitor, together with rotenone completely inhibited HEPG2 cell respiration and kept high J_m , while subsequent MitoQ₁₀ addition restored again respiration in an antimycin- (stigmatellin- and myxothiazol-)-dependent manner, but did not prevent high J_m . We conclude that MitoQ₁₀ is able to accept electrons prior to the rotenone-bound Q-site and that a reverse mode of Complex II is likely required to regenerate the reduced MitoQ₁₀H₂ back to MitoQ₁₀. Complex III inhibitors (antimycin, stigmatellin and myxothiazol) prevented high J_m in HEPG2 cells induced with either rotenone and MitoQ₁₀, or with rotenone, MitoQ₁₀, and thenoyltrifluoroacetone. MitoQ₁₀ alone increased basal J_m in HEPG2 cells highlighting also its *pro*-oxidant property. Described effects of all reagents combinations were more pronounced in J_m HEPG2 cells compared to H₂O₂ formation in isolated liver mitochondria. In conclusion, MitoQ₁₀ possesses a *pro*-oxidant role when added to intact mitochondrial respiratory chain, whereas its antioxidant role is striking when Complex I-derived superoxide generation increases due to retardation of electron flow within the Complex I.

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S10.20 Mitochondrial phospholipase iPLA2-dependent regulation of uncoupling protein UCP2

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We tested a hypothesis that reactive oxygen species (ROS)-dependent activation of mitochondrial phospholipases leads to an increase in respiration and to more intensive attenuation of mitochondrial ROS production due to UCP2-dependent uncoupling. This was tested using rat lung mitochondria and further verified using lung mitochondria isolated from UCP2-WT and UCP2-KO mice. Mitochondria with succinate as a substrate exhibited a steady increase in respiration which was further stimulated by low concentrations of *tert*-butyl hydroperoxide (TBHP, 5–25 μM). The observed respiration increase was fully inhibited by BSA, indicating the participation of free fatty acids, and by bromoenol lactone (BEL, 10 μM), a specific inhibitor of phospholipases iPLA2. The respiration was further partially inhibited by GTP, an inhibitor of UCP2. The TBHP-dependent increase in respiratory rate was not observed in lung mitochondria isolated from UCP2-KO mice. Parallel detection of H₂O₂ by Amplex Red revealed that neither BSA nor BEL caused a significant increase in mitochondrial H₂O₂ production under the given experimental

conditions. These results demonstrate the presence of iPLA2 in lung mitochondria and support the hypothesis that the activation of mitochondrial phospholipases by mild oxidative stress can provide free fatty acids as cycling substrates for UCP2. However, attenuation of ROS production by UCP2 is not significant.

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S10.21 Quinones inhibit the mitochondrial permeability transition pore at two sites

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We have studied the quinone structural features that confer modulatory properties on the mitochondrial permeability transition pore (PTP). Reduced derivatives of ubiquinone (Ub) 0 with acetoxy or methoxy substitutions of the carbonyl groups became ineffective at PTP inhibition. Consistent with a key role of the Ub0 oxidation-reduction state for its interactions with the PTP, DTT prevented the inhibitory effects on the pore when added before but not after Ub0. Of note, the addition of DTT after Ub0 prevented the toxic effects of Ub0 on respiration. The combination of Ub0 and DTT thus allowed inhibition of the PTP without mitochondrial toxicity, which in the absence of DTT reveals itself with a bell-shaped curve where the Ub0-dependent increase of mitochondrial Ca²⁺ retention capacity (a measure of PTP inhibition) is superceded by a decrease as the Ub0 concentration is raised above about 50 μM. The PTP inhibitory effects of decylUb were instead unaffected by reducing agents, and Ub0 and decylUb displayed additive effects on PTP inhibition, indicating that they act at different inhibitory sites on the PTP.

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S10.22 Novel mitochondria-targeted antioxidants are capable to defense cells from the oxidative damage

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Synthesized in the Skulachev's laboratory new type of mitochondria-targeted antioxidants — plastoquinone derivatives (SkQs) were investigated. The aim of our study was to estimate of antioxidant properties of the SkQ family members plastoquinonyl decyltriphenylphosphonium (SkQ1) and plastoquinonyl decylrhodamin 19 (SkQR1), a fluorescent derivative. These compounds combined with a positively-charged penetrating cations were selectively targeted to mitochondria, being accumulated inside, and can be reduced by the respiratory chain. Using confocal microscope we observed that SkQR1 was accumulated by mitochondria. Uncoupling action of FCCP, leading to mitochondrial depolarization, prevented the SkQR1 staining. In human cell cultures HeLa and K562 very low concentrations (nanomolar) of antioxidants were found to prevent ROS-induced apoptosis and necrosis. Oxidative stress initiated by addition of small amount H₂O₂ to living cells, caused secondary generation of endogenous ROS and lowered the level of reduced glutathione.

SkQ1 and SkQR1 prevented oxidative damage as well as glutathione oxidation, C1/2 being around 2nM and 0.5 nM respectively. It is concluded that cationic plastoquinone derivatives are rechargeable, mitochondria-targeted antioxidants of very high efficiency.

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S10.23 Variegated porphyria induces higher H₂O₂ production in stimulated lymphocytes due to an impaired respiratory function

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Our aim was to analyse the effects of VP on the production of ROS by lymphocytes and determine the possible mitochondrial sources of these ROS. Twelve women affected by VP and twelve control healthy women participated in the study. H₂O₂ production was measured using 2,7-dichlorofluorescein-diacetate as indicator in basal conditions and after stimulation with PMA. In addition three treatments with allopurinol, rotenone or myxothiazol were performed in PMA-stimulated lymphocytes. No differences were observed between porphyric and control women in the basal production of H₂O₂. The stimulation with PMA increased H₂O₂ production in both groups but lymphocytes from porphyric women produced higher levels of H₂O₂ than controls. The treatments with allopurinol and rotenone did not modify H₂O₂ production but after treatment with myxothiazol H₂O₂ production decreased back to basal levels in both groups. In conclusion, lymphocytes from women affected by VP produce the same amount of H₂O₂ in basal conditions than control women, but after stimulation lymphocytes from porphyric women produce higher levels of H₂O₂. This increased H₂O₂ production is due to an impaired function of the mitochondrial respiratory chain rather than to other ROS sources such as xanthine oxidase.

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S10.24 Involvement of p38 in camptothecin induced expression of Ucp2 in rat neonatal cardiomyocytes

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Camptothecin (CPT), a topoisomerase I inhibitor, is used for treatment of certain types of malignancies. Uncoupling protein 2 (Ucp2) is proposed to protect cardiomyocytes against oxidative stress. Modulation of Ucp2 level appears important because the protein increases in failing human heart. p38^{MAPK} belongs to a group of protein serine/threonine kinases that become activated in response to extracellular stimuli and mediate signal transduction in cell growth, differentiation and apoptosis. We found that CPT treatment induces Ucp2 expression on both mRNA and protein level in cardiomyocytes. This induction is accompanied by short-term increase in production of ROS (<1 h) preceded by activation of p38^{MAPK} (<30 min). Activation of p38^{MAPK} by CPT was comparable to anisomycin, a protein synthesis inhibitor that activates stress-related MAPKs, namely p38^{MAPK} in mammalian cells. Pretreatment of cardiomyocytes with p38^{MAPK} inhibitor, SB203580, blocked activation of p38^{MAPK} by both compounds and abolished the camptothecin-mediated Ucp2 induction. Our results