Mitochondria are the major source of reactive oxygen species (ROS) during the ischemia/reperfusion (I/R) and the same time one of the most susceptible compartment to the ROS damage. Oxidative stress and mitochondria dysfunction are believed to be main reasons of acute kidney injury after I/R. Inhibition of ROS production inside the mitochondria could protect these organelles from dysfunction during I/R and thus prevent cell death. For this purpose a new type of antioxidant molecules was designed. Due to the delocalized positive charge the antioxidant accumulates in mitochondrial matrix in concentrations highly exceeding its concentration in the cytosol or in the intracellular space. The aim of the work was to investigate the ability of mitochondria-targeted antioxidant 10-(plastoquinonyl)-decylrhodamine (SkQR1) to prevent oxidative stress and protect kidney from acute kidney injury on the model of 40-min ischemia of rat kidney. For the evaluation of ROS production and mitochondria membrane potential after I/R, DCF- and TMRE-loaded renal cortex tissue slices were analyzed by confocal microscopy. Malonic dialdehide (MDA)-products in kidney, blood urea nitrogen (BUN) and creatinine level were investigated 48 h after I/R. Histological study of renal tissue was also held. It was revealed that 40-min I/R led to the burst of ROS production, decrease of mitochondria membrane potential and fragmentation of mitochondrial reticulum. creatinine and BUN level increased 48 h after I/R about 4 and 6 times, respectively; MDA-products increased more than twice. Tubular lesions were observed by histological examination. Intraperitoneal injection of SkQR1 before I/R partly normalized ROS production and prevented mitochondria damage. The MDA-products in kidney were diminished. Administration of SkQR1 had a beneficial effect on kidney function: creatinine and BUN level decreased and there were minimal pathological changes in the kidney. We conclude that mitochondria-targeted antioxidant SkQR1 was able to normalize mitochondria functioning during I/R, prevent oxidative stress and having beneficial effect on acute kidney injury.

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5P.9 Effect of flavonolignans derived from silybin on mitochondrial production of reactive oxygen species

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Flavonolignans exert cytoprotective and anticancer effects generally ascribed to their antioxidant properties. We have tested seventeen chemically modified derivatives of the naturally occurring silybin and two derivatives of the flavonol quercetin to assess their alleviating effect on mitochondrial reactive oxygen species (ROS) production. We have used isolated intact rat heart mitochondria (RHM) and detected the mitochondrial production of reactive oxygen species using the Amplex Red assay for fluorometric monitoring of H_2O_2. Silybin titration (0.01–20 μM) resulted in only a mild decrease in the mitochondrial ROS production. The most prominent decrease in the detected ROS production was found in the case of 2,3-dehydrosilybinic acid, 2,3-dehydrosilybin (DHS) and its 3-*O*-methyl, 7-*O*-galloyl and 23-*O*-galloyl derivatives. *O*-Methylation at a position seven reverted the anti-oxidant effect of 3-*O*-methyl-2,3-dehydrosilybin. The half-maximum inhibitory concentration of DHS was found to be 0.15 mM. Moreover, the detection of mitochondrial respiration and membrane potential indicated that DHS and other tested compounds which decrease the mitochondrial ROS production also uncouple oxidative phosphorylation, an effect analogous to that of the synthetic uncoupler FCCP. In addition, we found DHS and its derivatives to be more effective uncouplers than quercetin. The similarity of the behavior between FCCP, DHS and selected derivatives suggests a direct protonophoretic mechanism. In summary, our data support previous studies indicating the ability of several bioflavonoids to uncouple respiration. These data further extend our previous results showing that DHS and several of its derivatives are more potent scavengers of ROS than silybin and we attribute these effects to their innate uncoupling properties.

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5P.10 Proton transfer and reactive oxygen species in the cytochrome bc_1 complex

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The mitochondrial cytochrome bc_1 complex links electron transfer from ubiquinol to cytochrome *c* by a protonmotive Q cycle mechanism in which ubiquinol is oxidized at center P and ubiquinone is reduced at center N [1,2]. E272 of the conserved PEWY loop of most cytochrome *b* has been suggested as ligand in the enzyme–substrate complex and as proton acceptor in parallel proton-electron transfer towards heme b_L [3]. E272D and E272Q mutations support the importance of the residue for correct ubiquinol oxidation, showing effects such as lowered ubiquinol cytochrome *c* reductase activity, elevated bypass reactions, and altered K_M for ubiquinol oxidation [4]. However, these effects may also be indirect and the role of E272 as direct ligand of ubiquinol is debated. Furthermore, E272 is not fully conserved across all species. We suggested that in *Beta-* and *Gamma-proteobacteria* of which the PEWY glutamate is substituted by valine or leucine, a glutamate equivalent to yeast H253 is conserved, which could take over the proton transfer function. To challenge this hypothesis, single and double substitutions of H253 and E272 have been constructed in *Saccharomyces cerevisiae*. Eight variants were produced and the detergent-solubilized and purified complexes were characterized. The mutations affect cytochrome *c* reductase activity and provoke reactive oxygen species production. Mechanistic implications for ubiquinol oxidase and the control of deleterious bypass reactions will be discussed.

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5P.11 ATP concentration change in *Caenorhabditis elegans*

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