9L.3 Mitochondrial generated nitric oxide protects against permeability transition *via* formation of membrane protein S-nitrosothiols

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Mitochondria generated nitric oxide (NO) regulates several cell functions including energy metabolism, cell cycling, and cell death. Here we report that the NO synthase inhibitors (l-NAME, l-NNA and l-NMMA) administered either *in vitro* or *in vivo* induce Ca^{2+}-dependent mitochondrial permeability transition (MPT) in rat liver mitochondria *via* a mechanism independent on changes in the energy state of the organelle. MPT was determined by the occurrence of cyclosporin A sensitive mitochondrial membrane potential disruption followed by mitochondrial swelling and Ca^{2+} release. In *in vitro* experiments, the effect of NOS inhibitors was dose dependent (1 to 50 μM). In addition to cyclosporin A, l-NAME induced MPT was sensitive to Mg^{2+} plus ATP, EGTA, and to a lower degree, to catalase and dithiothreitol. In contrast to l-NAME, its isomer d-NAME did not induce MPT. l-NAME induced MPT was associated with a significant decrease in both the rate of NO generation and the content of membrane protein S-nitrosothiol. Acute and chronic *in vivo* treatments with l-NAME also promoted MPT and decreased the content of mitochondrial protein S-nitrosothiol. SNAP (a NO donor) prevented l-NAME mediated MPT and reversed the decrease in the rate of NO generation and in the content of membrane protein S-nitrosothiol. We propose that S-nitrosylation of critical membrane protein thiols by NO protects against MPT.

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Posters

9P.1 Stigmatellin as a modulator of metal-induced inner mitochondrial membrane permeabilization

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Previously on two rat cell lines, AS-30D and PC12, we have shown that stigmatellin (an inhibitor of mitochondrial respiratory complex III) is one of the strongest protectors against Cd^{2+}-induced cytotoxicity, in addition to N-acetylcysteine and several mitochondrial permeability transition (MPT) pore inhibitors, namely bongkrekic acid (BKA) and cyclosporine A (CsA). To better understand the molecular mechanisms of the preventive action of stigmatellin, we tested its effectiveness against mitochondrial membrane permeabilization produced by such heavy metals as Cd, Hg, Cu, and Zn, as well as by Ca (in the presence of Pi) or Se (added as Na_2SeO_3), using isolated rat liver mitochondria as a model system. The conducted experiments showed that stigmatellin exhibited the modulating effects on the mitochondrial swelling induced by these metals/metalloids in isotonic sucrose medium in the presence of Asc and TMPD (complex IV substrates) added for energization of the mitochondria in order to bypass the respiratory complexes I, II, and III inhibited by Cd^{2+} etc. In particular, stigmatellin sharply enhanced the mitochondrial swelling, evoked by selenite; however, in the same medium and under the same conditions stigmatellin as well as BKA and CsA did not produce significant effect on Cu^{2+}-induced swelling of isolated rat liver mitochondria in contrast to the high-amplitude swelling produced by Cd^{2+}, Hg^{2+}, Zn^{2+}, or Ca^{2+} plus Pi, which significantly depressed by these inhibitors. In the light of own results and data from literature obtained during the latest time, the hypothesis suggested by us earlier (Belyaeva (2004) Mitochondrion 4: 71; Belyaeva et al., (2004) Chem.-Biol. Interact. 150: 253–270) about the possible involvement of the electron transport chain supercomplex, formed by complex I (P-site) and complex III (S-site) in the mitochondrial membrane permeabilization mediated by the MPT pore is discussed.

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9P.2 MitoTeas: *Vaccinium myrtillus* and *Geranium robertianum* decoctions improve diabetic Goto–Kakizaki rats hepatic mitochondrial oxidative phosphorylation

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Diet-induced metabolic syndrome, leading to obesity, insulin resistance, type 2 diabetes and related diseases are major health problems all over the world, nowadays [1,6]. A common feature to these metabolic alterations is lower mitochondrial oxidative phosphorylation (OXPHOS) enzymatic complexes activities [5]. Several chemical compounds found in plant products had proven to possess beneficial properties, being currently pointed out due to their pharmacological potential in metabolic syndrome complications [2]. In this context, we studied the effect of *Vaccinium myrtillus* and *Geranium robertianum* leaf decoctions on Goto-Kakizaki (GK) rats, a type 2 diabetes mellitus animal model. Our results show that *V. myrtillus* and *G. robertianum* leaf decoctions present significant benefits on glycaemic control and that GK rats treated during four weeks with *V. myrtillus and G. robertianum* decoctions presented an improvement of the evaluated mitochondrial respiratory parameters (state 3, state 4, RCR and FCCP stimulated respiration). These increased OXPHOS activities can be correlated to the high contents of quercetins found in *V. myrtillus* and homoeriodictyol found in *G. robertianum,* that are reported to account for increased protein expression [3,4]. Therefore, these "MitoTeas" seem to be promising therapeutic agents to type 2 diabetes, regarding their high antioxidant activity coupled to their beneficial effects on glycaemic control and mitochondrial activity.

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9P.3 A novel drug for uncomplicated malaria: Targeted high throughput screening (HTS) against the type II NADH:ubiquinone oxidoreductase (PfNDH2) of *Plasmodium falciparum*

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The respiratory chain of the human malaria parasite *Plasmodium falciparum* lacks a canonical protonmotive NADH:ubiquinone oxidoreductase (Complex I), containing instead a single-subunit, non-protonmotive NDH2, similar to that found in plant mitochondria, fungi and some bacteria [1,2]. As such, the *P. falciparum* NDH2 (PfNDH2) presents itself as an attractive anti-malarial chemotherapeutic target, and we have developed a heterologous expression system for this enzyme in the *E. coli* NADH dehydrogenase knockout strain ANN0222 (generously provided by Prof. Thorsten Friedrich, Freiburg) to facilitate its physicochemical and enzymological characterisation [3]. PfNDH2 represents a metabolic choke point in the respiratory chain of *P. falciparum* mitochondria and is the focus of a drug discovery programme towards the development of a novel therapy for uncomplicated malaria. Here we describe a miniaturised assay for recombinant PfNDH2 with robust assay performance measures that has been utilised for the high throughput screening (HTS) of small molecule inhibitors. The objectives of the HTS were to (i) increase the number of selective PfNDH2 inhibitors and (ii) to expand the number of inhibitor chemotypes. At the time of screening, only one proof of concept molecule, 1-hydroxy-2-dodecyl-4-(1H)quinolone (HDQ), was known to have PfNDH2 inhibitory activity ($IC_{50} = 70$ nM) [3,4]. This molecule was used to initiate a primary similarity-based screen of 1000 compounds from a compound collection of 750 000 compounds (curated by Biofocus-DPI). A range of chemoinformatics methods and filters were applied to the hits from this initial phase in order to perform a hit expansion screen on a further about 16 000 compounds. The chemoinformatic strategy allowed us to cover about 16% diversity whilst screening just about 2% of the compound collection. The HTS resulted in a hit rate of 0.29% and 150 compounds were progressed for potency against PfNDH2. Of these compounds, 50 were considered active with IC_{50}s ranging from 100 nM to 40 µM. Currently, seven distinct chemotypes are being progressed from hit to lead using traditional synthetic medicinal chemistry strategies.

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9P.4 Mitochondrial function and idebenone: A good therapy for Leber's hereditary optic neuropathy?

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Idebenone [2,3-dimethoxy-5-methyl-6(10-hydroxidecyl)-1,4-benzoquinone] is a synthetic analogue of coenzyme Q10 (CoQ10), an essential constituent of the mitochondrial electron transport chain and a powerful antioxidant. Idebenone is also a good electron carrier in the mitochondrial respiratory chain. Quinones (including idebenone) have also been shown to affect the mitochondrial permeability transition (PT) pore (PTP) a high-conductance inner membrane channel modulated by the proton electrochemical gradient and by many signaling molecules. PTP links oxidative stress to cell death and seems to be involved in Leber's hereditary optic neuropathy (LHON) and other pathologies of neurological interest. Given these complex effects of idebenone on cellular bioenergetics we have investigated its effects on bioenergetics and PTP modulation in intact cells. Our preliminary results indicate that: (i) idebenone modulates the PTP *in situ* through an interaction with NEM-sensitive thiols, with an effect that can be inhibited by Cyclosporin A (CsA); (ii) DTT prevents the PTP-inducing effects of idebenone, and promotes electron transfer from idebenone to complex III of the respiratory chain bypassing the lack of complex I activity; (iii) in the presence of DTT, idebenone considerably increases antimycin A-sensitive respiration both in normal and in RJ206 cells (harboring the 3460/ND1 LHON mutation) and XTC.UC1 thyroid oncocytoma cells (bearing a disruptive frameshift mutation in the MT-ND1 gene which impairs complex I assembly). The key question of whether idebenone-supported respiration is used for ATP synthesis is being addressed.

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9P.5 Dietary supplementation with docosahexaenoic acid, but not eicosapentanoic acid, remodels cardiac mitochondrial phospholipid fatty acid composition and prevents permeability transition

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Treatment with the ω-3 polyunsaturated fatty acids (PUFAs) docosahexanoic acid (DHA) and eicosapentanoic acid (EPA) exerts cardioprotective effects in patients, and suppresses Ca^{2+}-induced opening of the mitochondrial permeability transition pore (MPTP) *in vitro*. These effects are associated with increased DHA and EPA and lower arachidonic acid (ARA) in cardiac phospholipids. ARA is implicated in inflammation and induction of MPTP opening. While clinical studies suggest the triglyceride lowering effects of DHA and EPA are equivalent, there is growing evidence that DHA may be superior at remodeling mitochondrial phospholipids and preventing MPTP. Therefore we compared the effects of dietary supplementation with the ω-3 PUFAs DHA and EPA on cardiac mitochondrial phospholipid fatty acid composition and Ca^{2+}-induced MPTP opening. Rats were fed either a control (CTRL) low-fat chow, or a similar diet supplemented with either DHA or EPA only at 2.5% of energy intake for 8 weeks. These doses of DHA and EPA are comparable to about 5 g/day in humans. Cardiac mitochondria were isolated and analyzed for Ca^{2+}-