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doi:10.1016/j.bbabio.2010.04.240

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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doi:10.1016/j.bbabio.2010.04.241

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.

doi:10.1016/j.bbabio.2010.04.242

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+}-

induced MPT, respiration, and phospholipid fatty acyl composition. Both DHA and EPA enriched diets lowered circulating free fatty acids and triglycerides by approximately 40% ($p<0.05$, DHA vs CTRL and EPA vs CTRL, NS, DHA vs EPA). DHA supplementation increased DHA by 63% ($p<0.05$ vs control) and decreased ARA by 61% ($p<0.05$ vs control) in mitochondrial phospholipids, and significantly delayed MPTP opening (57% more calcium necessary to induce MPTP vs CTRL, $p<0.05$). EPA supplementation did not affect DHA, only modestly lowered ARA (−33% vs CTRL, $p<0.05$), and had no effect of MPTP opening. State 3 respiration with a variety of substrates was unaffected by dietary treatment, however DHA decreased state 4 respiration by 30% and the increased RCR by 70% with pyruvate + malate as the substrate, both in the absence and presence of oligomycin ($p<0.05$); treatment with EPA had no effect. The P:O ratio was not different among groups with any of the substrates. In summary, DHA supplementation favorably altered mitochondrial phospholipid composition and delayed MPT in cardiac mitochondria, while EPA had no effect. These effects may contribute to the protection against heart disease with ω-3 PUFA supplementation, and suggest that supplementation with DHA should be superior to EPA.

doi:10.1016/j.bbabio.2010.04.243

9P.6 Nitrolinoleate modifies ANT, K_{ATP} channels and complex II and modulates their activity

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Nitroalkenes are electrophilic molecules which can cause post-translational modifications of proteins and modulate their functional activity. Previously we demonstrated endogenous formation of nitrated linoleate (LNO_2) in mitochondria isolated from perfused heart after ischemic preconditioning. In addition, synthetic LNO_2 protected isolated cardiomyocytes against simulated ischemia/reperfusion injury. Biotin-tagged LNO_2 replicated this cardioprotective effect, and caused reversible modification of mitochondrial proteins. Thus, we hypothesized that mitochondrial targets of LNO_2 might play an important role in cardioprotection. Previously we demonstrated that LNO_2 induced mitochondrial H^+ leak *via* modification of ANT. Further studies revealed that LNO_2 (1 μM) opened mitochondrial K_{ATP} channels in a 5-HD and glybenclamide sensitive manner. Although the molecular identity of the mK_{ATP} channel has not been fully elucidated, we previously showed that complex (Cx) II might be involved in regulation of mK_{ATP} channel activity. We found that LNO_2 physically interacted with the 70 kDa subunit of Cx II and inhibited its enzymatic activity. Notably, the cardioprotective effects of mild H^+ leak, opening of mK_{ATP} channels and reversible inhibition of the respiratory chain are well documented. Thus, our findings characterize LNO_2 as a pleotropic molecule which might recruit several protective mitochondrial pathways to elicit cardioprotection.

doi:10.1016/j.bbabio.2010.04.244

9P.7 Doxorubicin-induced cardiac, hepatic and renal mitochondrial toxicity in an acute *versus* sub-chronic treatment model

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Nowadays, Doxorubicin (DOX) is probably one of the most effective anticancer drugs available in the clinic. However, the treatment is usually followed by a cumulative and persistent cardiotoxicity. Mitochondria have a critical role in DOX-mediated toxicity however there are still doubts whereas mitochondrial toxicity is specific to the heart. Therefore, the present work characterizes two different models of toxicity (acute vs. sub-chronic), regarding mitochondrial physiology from three different tissues (heart, liver and kidney) from treated rats. Wistar rats were sub-chronically (7 wks, 2 mg/kg) or acutely (20 mg/kg) treated with DOX and allowed to rest one week or 24 h after the last injection, respectively. Sub-chronically-treated animals showed a decrease in body mass gain during treatment while no changes were observed in acute model. Plasma profile from both models was altered but the sub-chronic treatment presented the most dramatic changes. Histological analysis revealed the presence of lipid droplets in liver slices from acutely treated rats. Regarding mitochondrial bioenergetics, differences between saline and DOX-treated rats were observed: in the acute model, differences included state 3 respiration in the liver and kidney and the ADP/O in the heart. In the sub-chronic model, differences regarding state 3 respiration in the heart and kidney was observed. We also determined that cardiac mitochondria from sub-chronic-treated animals presented a lower calcium loading capacity, which was not observed in the other tissues. However, gene expression analyses showed no alterations in the chronic model but interestingly, decreased mRNA levels for the ANT, VDAC and increased CyP-D mRNA were detected in the acute model. Aconitase activity, a sensitive marker of oxidative stress, was decreased in the kidney (acute model) and in the heart (sub-chronic model). In conclusion, data confirm that mitochondrial alterations result from DOX treatment, being more severe in the heart and are very dependent on the treatment protocol. It remains to be determined if mitochondrial alterations in organs such as liver and kidneys are a specific and direct effect of DOX on mitochondria or if they result of secondary effects of DOX on other targets.

The present work is supported by the Portuguese FCT (SFRH/BD/36938/2007 to GP, PTDC/SAU-OSM/64084/2006 and PTDC/SAU-OSM/104731/2008 to PO).

doi:10.1016/j.bbabio.2010.04.245

9P.8 Glycine regulates calcium capacity of isolated brain mitochondria

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Glycine, inhibitory neurotransmitter, has been found to be effective against neuronal cell death in *in vivo* and *in vitro* models of ischemic stroke. We have shown that glycine prevented respiratory index depletion of mitochondria in the homogenate of the cerebral cortex after 24 h common carotid artery occlusion in rats, along with preventing the