Coupling Uncoupling: Deciphering the Molecular Symphony

Exploring the Impact of Mitochondria Uncoupling Chemicals on the Transcriptome and Metabolome of Zebrafish Embryos

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INTRODUCTION

Mitochondrial uncoupling chemicals manifest their effects by disrupting oxidative phosphorylation (OXPHOS), a main metabolic pathway that produces ATP. Uncoupling of OXPHOS can cause reduced ATP production and cell proliferation. This series of events are known to cause adverse effects of regulatory concern, such as growth inhibition (AOPWiki, AOP #263). This study aims to utilise new approach methodologies (NAMs), in particular the zebrafish embryo model as an alternative to fish testing, to generate in-depth systems biology and quantitative understanding to support AOP # 263.

In addition to targeted bioassays, a regulatory toxicity protocol, Fish Embryo Toxicity Test (FET, OECD TG 236), and multi-OMICS (transcriptomics and metabolomics) as a high-content NAM was used to generate new knowledge on the temporal and concentration-dependent stress response patterns in response to mitochondrial uncoupling at the whole-organism scale.

MATERIALS AND METHODS

Zebrafish embryos were exposed to different concentrations of the reference mitochondrial uncoupler carbonyl cyanide m-chlorophenyl hydrazone (CCCP) from 3 to 96 hours post fertilisation (hpf) in the format of a FET test.

Assays
- ATP (48, 72, 96 hpf)
- Growth (96 hpf)
- Chemical analysis

Omics (48,72, 96hpf, 5-80nM CCCP)
- Transcriptomics RNA-seq

→Differentially expressed genes (DEG) *DESeq2*
- Untargeted metabolomics LC/MS-MS

→Differentially expressed metabolites (DEM) *MetaboAnalyst*
- Gene set enrichment analysis

Pathway analysis
- Gene set enrichment analysis (GSEA) & common enriched pathways (temporal and dose-dependent) *GlueGO*
- Joint pathway analysis of DEGs and metabolites *Metaboanalyst*

RESULTS & CONCLUSION

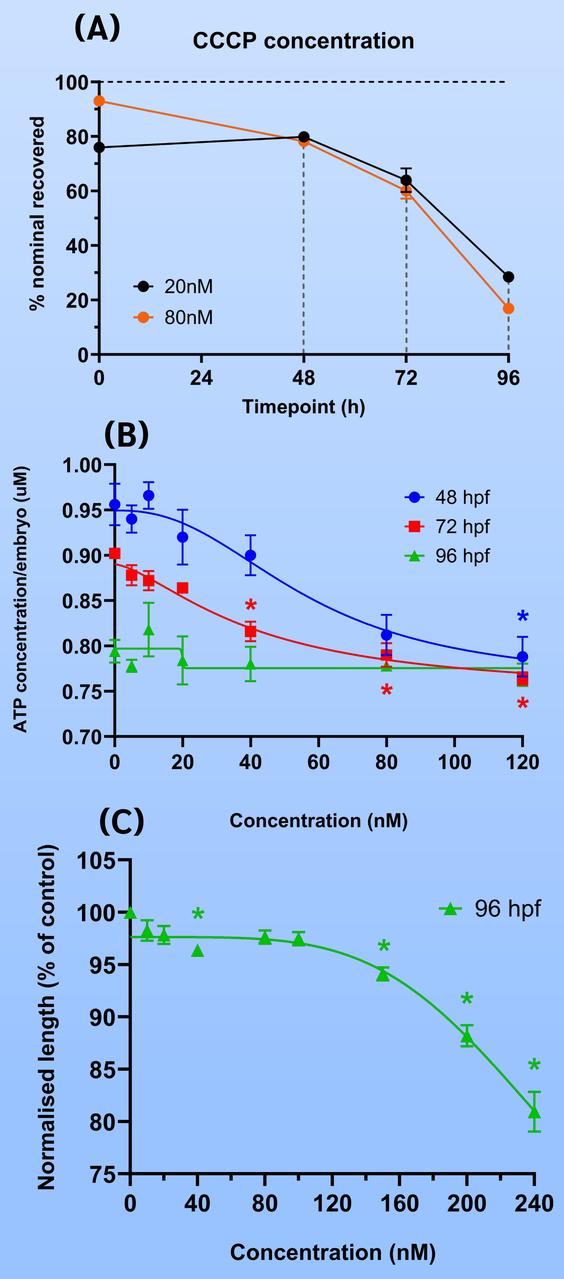

Figure 1: Chemical analysis of exposure media (A) and effects of CCCP on ATP (B) and length (C) of zebrafish embryos. Data are mean ± standard error. Asterisks indicate significant difference from control ($p < 0.05$).

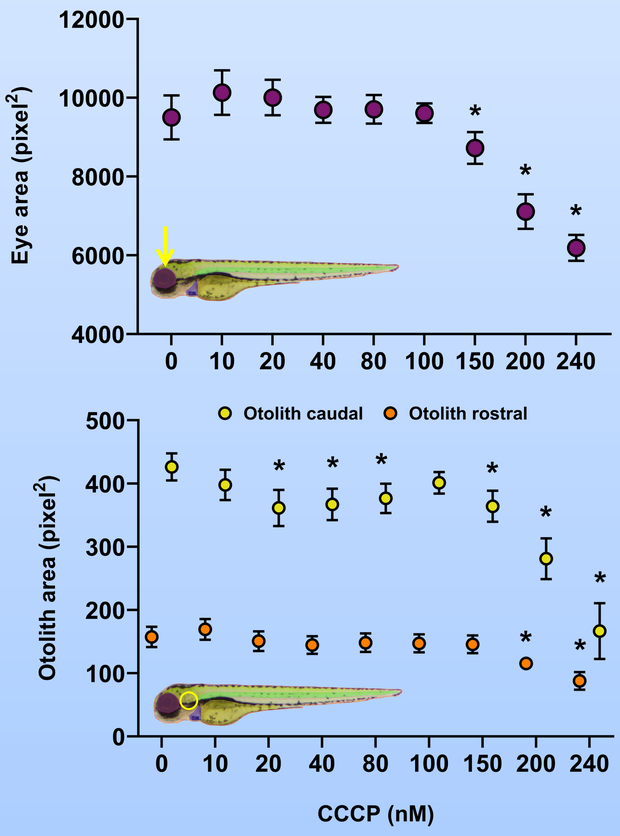

Figure 2. Effects of exposure to CCCP for 96h on eye and otoliths area of zebrafish larvae. Asterisks indicate significant differences compared to control ($p < 0.05$). Data shown are means ± 95% CI.

Table 1: Joint pathway analysis of DEGs and metabolites of zebrafish embryos exposed to 80nM CCCP until 96 hpf. Gene hits: total number of DEGs involved in pathway. Compound hits: total number of metabolites involved in pathway. FDR: false discovery rate.

Pathways	Gene hits	Compound hits	FDR
Retinol metabolism	16	2	4.00E-04
Glycine, serine and threonine metabolism	17	0	0.0011
Drug metabolism - other enzymes	21	0	0.0011
PPAR signaling pathway	22	0	0.0012
Steroid hormone biosynthesis	13	3	0.0061
ECM-receptor interaction	23	0	0.0061
Tryptophan metabolism	16	0	0.0061
Arginine and proline metabolism	16	1	0.0127
Drug metabolism - cytochrome P450	12	0	0.0152
Histidine metabolism	10	0	0.0156
Primary bile acid biosynthesis	9	0	0.0164
Amino sugar and nucleotide sugar metabolism	15	2	0.0164
Starch and sucrose metabolism	12	0	0.0185
Peroxisome	21	0	0.0189
Metabolism of xenobiotics by cytochrome P450	12	0	0.0209
Pentose and glucuronate interconversions	8	1	0.025

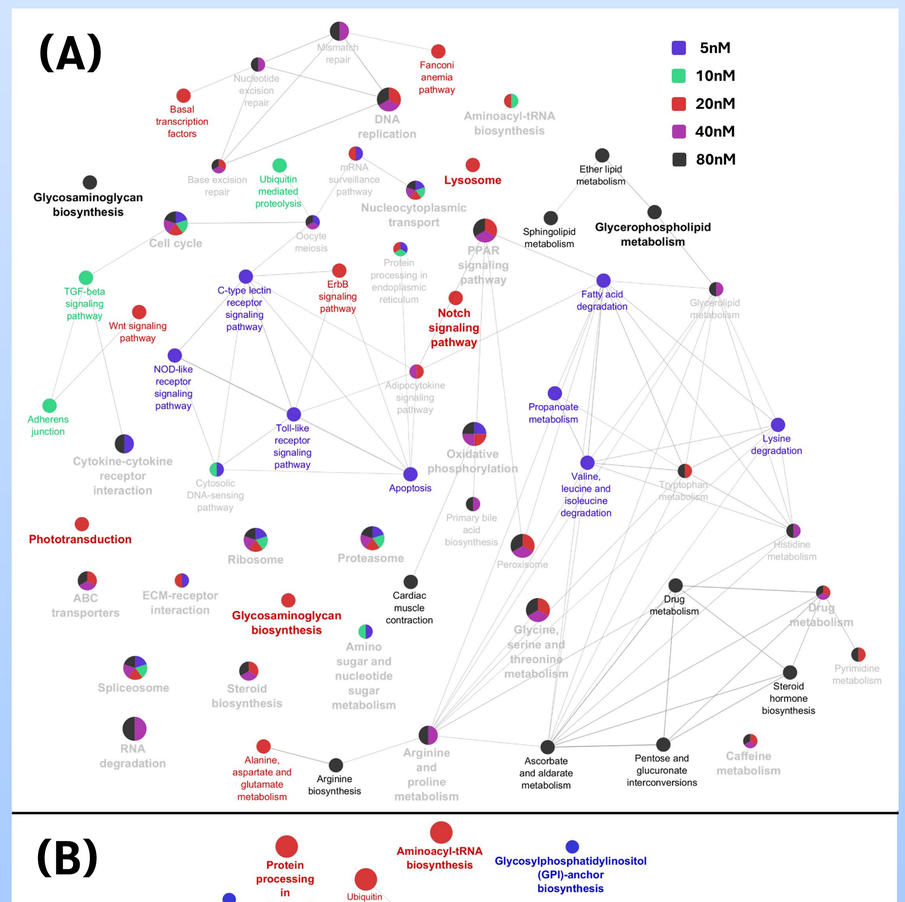

Figure 3: Pathway networks showing the common enriched pathways at different concentrations of CCCP at 96hpf (A), and the same concentration of CCCP (80nM) at different timepoints (B).

CCCP exposure disrupted the oxidative phosphorylation pathway in a temporal and concentration-dependent manner in zebrafish embryos (Fig 3A,B) affecting the energy reserves (ATP, Fig 1B) and leading to growth inhibition in terms of length (Fig 1C), eye and otoliths area (Fig 2). Joint pathway analysis as well as GSEA analysis revealed affected pathways involved in metabolism and cell proliferation e.g. PPAR signaling pathway and retinol metabolism (Table 1, Fig. 3). Work is ongoing to identify possible biomarkers and pathways to support an Adverse Outcome Pathway network on mitochondrial uncoupling (OECD AOP project 1.92, AOPWiki AOP 263-268).

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