Towards A Quantitative Systems Pharmacology Approach to Evaluate the Anti-Obesity Potential of Oxidative Phosphorylation Uncouplers

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Objectives: To characterize the pharmacokinetic and pharmacodynamic endpoints of the prototypical oxidative phosphorylation uncoupler 2,4-dinitrophenol (DNP) \textit{in vitro} and to develop a QSP model to evaluate the potential of oxidative phosphorylation uncouplers as anti-obesity agents.

Methods: We investigated the \textit{in vitro} pharmacokinetics and pharmacodynamics using DNP as a prototypical uncoupler in differentiated, murine 3T3-L1 adipocytes. The stability and intracellular concentrations of DNP were quantified using LC-MS/MS methods. Cellular metabolic processes including oxygen consumption rate, proton leak, basal respiration and ATP production were measured using a Seahorse XFe96 Analyzer Mito Stress Test. In addition, total ATP, triglyceride content and membrane potential were measured via fluorescence. Finally, a QSP model was developed to fit the observed data.

Results: DNP was found to be stable in cell culture media and intracellular concentrations of DNP were ten-fold lower than media concentrations. 50 µM DNP was able to elicit a 40% reduction in triglyceride content and 22% decrease in ATP compared to control cells with continuous exposure. In addition, 50 µM DNP decreases mitochondrial membrane potential and increases oxygen consumption 3- 4 fold from baseline.

Conclusions: The results of this study suggest that the effects of DNP are both concentration and time dependent. A 50 µM concentration of DNP was sufficient to decrease membrane potential, increase oxygen consumption and reduce ATP and triglyceride content following continuous exposure. We successfully developed a QSP model that characterized the data well and predicts the optimal exposure-response relationship of DNP for triglyceride reduction \textit{in vitro}. This model will be useful for translating preclinical information to make predictions about weight loss in a clinical setting.