



Dose-dependent Response of HepG2 Liver Cells to the Mitochondrial Uncoupler FCCP

Research Area

Mitochondrial Physiology

Application

Mitochondrial Function:
How to inject and measure multiple doses of a mitochondrial uncoupler

Cell Type

Human Liver Cell Line (HepG2)

Work Of

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Tool Box:

- FCCP

Keywords:

- dose response
- FCCP
- HepG2 liver cells
- mitochondria
- oxidative phosphorylation
- respiratory capacity
- uncoupled respiration

Mitochondrial respiration is composed of coupled and uncoupled respiration. Uncoupling agents such as FCCP abolish the obligatory linkage between the respiratory chain and the phosphorylation system used to generate ATP. In response to increasing concentrations of uncoupling agents, cells will eventually reach a maximal metabolic rate thus defining their respiration capacity under a set of conditions. In this case study, the dose response of the human liver cell line, HepG2, to FCCP was assessed.

Background

Mitochondria generate 95% of cellular ATP via oxidative phosphorylation and are central to intermediary metabolism, free radical generation, and regulating apoptosis. Loss of mitochondrial function is tolerated by most cells until a threshold is reached when lack of ATP generation potential endangers the cell. Glycolytic flux accelerates to compensate, but this is finite, and at some point, which is different for different cells, the cell will die via necrosis or apoptosis. Their crucial role in maintaining cell viability and in many other metabolic pathways renders mitochondrial integrity a key component of cell physiology.

Measuring Bioenergetics

Valuable insight into the physiological state of living cells and the changes of those cells in response to experimental intervention has been gained through monitoring independently the rate of oxygen consumption using Clark electrodes or the rate of extracellular acidification using the microphysiometer. Under typical in vitro cell culture conditions, oxygen consumption rate (OCR) is a direct measurement of mitochondrial respiration and extracellular acidification rate (ECAR) is dominated by lactic acid production formed during glycolytic energy metabolism. Measuring both parameters simultaneously enables a more comprehensive assessment of cellular energetics and the ability to determine the dynamic interplay between these two dominant energy yielding pathways. Recognition of the value of measuring OCR and ECAR is underscored by the growing number of investigators using the Seahorse XF24 to achieve this dual metabolic measurement.

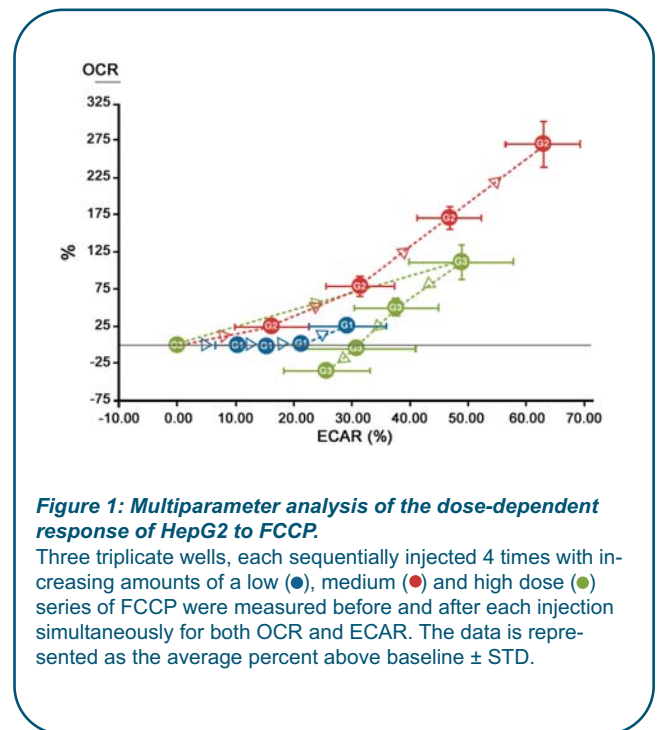


Figure 1: Multiparameter analysis of the dose-dependent response of HepG2 to FCCP.

Three triplicate wells, each sequentially injected 4 times with increasing amounts of a low (●), medium (●) and high dose (●) series of FCCP were measured before and after each injection simultaneously for both OCR and ECAR. The data is represented as the average percent above baseline \pm STD.

Application Note:

Dose-dependent Response of HepG2 Liver Cells to the Mitochondrial Uncoupler FCCP

Materials & Methods

Cell Culture.

Human hepatoma cell line, HepG2 was obtained from ATCC. Cells were cultured in growth medium consisting of DMEM, 10% FBS, 2mM GlutMax, 1mM Sodium Pyruvate and 100µg/mL Penicillin-Streptomycin. All cultures were maintained at 80% to 90% confluence at the time of subculture.

Test Compounds.

Carbonylcyanide-4-trifluoromethoxyphenylhydrazone (FCCP) was obtained from Sigma (St. Louis, MO). Concentrated stocks of 1 mM FCCP was prepared in DMSO and then diluted in assay media and the pH adjusted to 7.4.

References

Wu, M et al. (2007). Multiparameter metabolic analysis reveals a close link between attenuated mitochondrial bioenergetic function and enhanced glycolysis dependency in human tumor cells. *Am J Physiol Cell Physiol* **292**:C125-C136

Sridharan, V et al. (2007). The prolyl hydroxylase oxygen-sensing pathway is cytoprotective and allows maintenance of mitochondrial membrane potential during metabolic inhibition. *Am J Physiol Cell Physiol* **292**:C719-C728.

Dose response of HepG2 liver cells to FCCP

Compounds that dissipate the mitochondrial membrane potential uncouple the electron transfer system from adenylate phosphorylation, causing an increase in OCR and ECAR as the cell tries to reestablish the electrochemical gradient through increased electron flux. This is demonstrated in Figure 1 by exposing the human liver cell line, HepG2, to the uncoupler, carbonylcyanide-p-trifluoromethoxyphenylhydrazone (FCCP). In Figure 1, cells were exposed to increasing concentrations of FCCP that were injected automatically during the experiment. Plotting the OCR and ECAR responses together produced a bioenergetic chart indicative of both mitochondrial respiration and glycolysis, respectively. By injecting sequentially three dose series containing four escalating concentrations of FCCP, low (0.01 µM, 0.03 µM, 0.05 µM, 0.1 µM), medium (0.2 µM, 0.4 µM, 0.8 µM, 1.6 µM) and high (3.2 µM, 6.4 µM, 12.8 µM, 25.6 µM), the minimum, maximal and toxic responses were easily determined in 9 wells of a microplate.

Figure 2 shows only the mitochondrial component of the data, OCR, versus FCCP concentration. After the maximal response is reached, higher concentrations become acutely toxic to the cells resulting in decreased OCR. The ability to inject up to four compounds into each well facilitates generation of dose curves and EC50 values; the data for Figure 1 and 2, are derived from 9wells and took 1.5 hours.

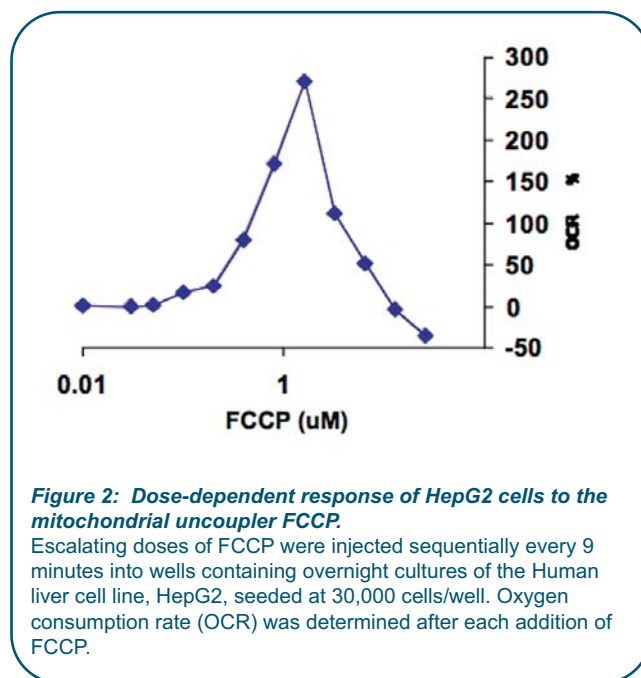


Figure 2: Dose-dependent response of HepG2 cells to the mitochondrial uncoupler FCCP.

Escalating doses of FCCP were injected sequentially every 9 minutes into wells containing overnight cultures of the Human liver cell line, HepG2, seeded at 30,000 cells/well. Oxygen consumption rate (OCR) was determined after each addition of FCCP.