HEPATOTOXICITY PREDICTION
How can you spot early adverse effects involving mitochondria?

Efficient, rapid and affordable in vitro tests confirm mitochondrio-toxic effects of three well-known hepatotoxic drugs. Learn more about our process, available as a robust predictive test suitable for compound screening.

Early detection of drug-induced hepatotoxicity is of major concern for pharmaceutical companies, as it is a frequent cause of interruption of clinical trials or market withdrawal. Mitochondrial dysfunction plays a major part in liver injury due to drastic metabolic perturbations and induced cell death. However, adverse effects involving mitochondria remain little investigated. Mitologics upgraded its established MiToxView® platform by developing a competitive range of tests for adverse effects on hepatic mitochondria using Seahorse technology.

Three drugs with known mitochondrial toxicity (Acetaminophen, Acetylsalicylic acid, and Amiodarone) were used to demonstrate the capacity of our system. The effects of these drugs on hepatic mitochondria were confirmed as we recorded a strong inhibition of spare respiratory capacity. Our process is thus suitable for rapid (1-week experimental workflow), effective and affordable compound testing/screening, to allow the selection of more promising and more valuable Leads

INTRODUCTION

Drug-induced liver injury can induce acute or chronic diseases, leading to long-term hospitalization or patient death in the worst scenario. Consequently, it is a major issue for public health and pharmaceutical companies. Indeed, because of their extensive first-pass hepatic extraction, many drugs accumulate in the liver, and especially in hepatic mitochondria, which favors their interactions with subsequent targets (Begriche et al., 2011). As a result, mitochondrial dysfunction is a key mechanism triggering liver injury, linked to altered energy production or the release of pro-apoptotic factors. Most drugs withdrawn from the market, or with Black Box warnings from the FDA, have effects on mitochondria (Dykens and Will, 2007). Thus, assessing mitochondrial toxicity could reliably eliminate the most dangerous drugs during preclinical safety studies, while also shedding light on the underlying mechanisms (Will and Dykens, 2014).
In this work, three drugs known to trigger mitochondrial alteration (Acetaminophen, Acetylsalicylic acid, and Amiodarone) were investigated using a Seahorse XFe96 and MiToxView® screening platform to illustrate the performance of our assays in predicting drug-induced toxicity.

HOW CAN WE HELP YOU?

- For your test compounds, similar studies can be performed in few weeks at the screening, Lead optimization, or preclinical stages.
- Contact Mitologics to discuss your needs. info@mitologics.com

CONTRIBUTORS TO THE STUDY

- **AGILENT TECHNOLOGIES INC.** (USA) provided the XF Cell Mito Stress Test kit
- **BIOPREDIC INTERNATIONAL** (France) provided HepaRG® cells
- **MITOLOGICS SAS** (France) was responsible for study design, tests on the Seahorse XFe96, data analyses and interpretation, as a part of the classical MiToxView® service

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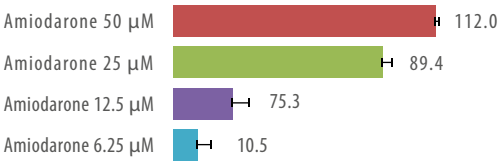
RESULTS

Fig 1: **Amiodarone strongly inhibits spare respiratory capacity.** a/ Dose-response inhibition of spare respiration capacity by Amiodarone and b/ Seahorse profile of HepaRG cells after 4 h treatment with four concentrations of Amiodarone using the Mito Stress Test kit (Oligomycin, FCCP, Rotenone/Antimycin A) (means +/- SEM of four experiments).
OCR: oxygen consumption rate;
Spare respiratory capacity = Maximal respiration (after FCCP) – basal measurement



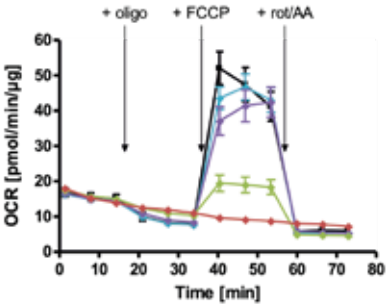
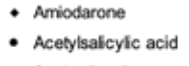
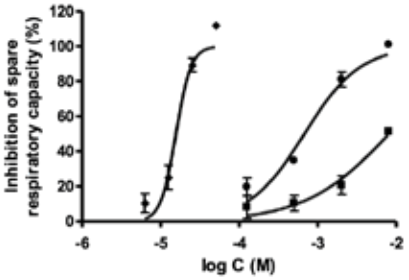


Fig 2: **Amiodarone, Acetylsalicylic acid and Acetaminophen, three hepatotoxic compounds, strongly inhibit spare respiratory capacity.** a/ Dose-response inhibition of spare respiratory capacity by Amiodarone, Acetylsalicylic acid and Acetaminophen on HepaRG cells (means +/- SEM of four experiments) and b/ Table of calculated EC20 for the three mitotoxic agents.

	EC_{20} (µM)
Amiodarone	**11.56**
Acetylsalicylic acid	**218.27**
Acetaminophen	**1472.31**





ADDITIONAL INFORMATION

Biopredic

HepaRG® cell line by Biopredic International
The human hepatoma HepaRG® cell line (HPR116, Biopredic International) differentiates into hepatocyte-like cells expressing xenobiotic metabolizing activities close to those measured in primary human hepatocyte cultures (Guillouzo et al., 2007), and proved to be a relevant model to investigate toxicity toward hepatic mitochondria (Porceddu et al., 2018). HepaRG cells display high levels of spare respiratory capacity, allowing sensitive detection of respiratory chain inhibition when screening compounds.

Agilent

Seahorse Analyzer
Agilent's Seahorse XFe96 Analyzer can be used to measure in real-time oxygen consumption in live cells with solid-state sensor probes. Data can be used to calculate the oxygen consumption rate (OCR). An integrated drug delivery system allows sequential addition of up to four compounds to each well at user-defined intervals. The XF Cell Mito Stress Test kit provides reference modulators of mitochondrial respiratory chains and reveals key parameters of mitochondrial function: basal, ATP-linked and maximal respiration.

Mitologics

Consultancy & Tests
The MiToxView® platform consists in in vitro methods assessing both the integrity and functionality of purified mitochondria (Porceddu et al., 2012) as well as cellular and mitochondrial alterations occurring in cultured cells (Porceddu et al., 2018). Multiparametric assays by spectrofluorimetry and Seahorse analysis are performed at Mitologics, applying Standard Operating Procedures to provide robust and reliable detection of compounds' mitochondrial-toxicity.

Information on the drugs tested
Acetaminophen (paracetamol) is commonly used to treat pain and fever. It is known to be transformed into the reactive metabolite NAPQI that directly targets the respiratory chain to inhibit mitochondrial ATP synthesis and oxygen consumption. Acetaminophen also induces glutathione depletion and peroxynitrite formation, leading to mtDNA damage.
Acetylsalicylic acid is commonly used to treat pain and fever due to various causes. Mitochondrial toxicity induced by acetylsalicylic acid is linked to CoA sequestration, leading to impaired ß-oxidation of fatty acids. This drug also triggers mitochondrial uncoupling at low concentrations (mitochondrial permeability transition (MPT)), and cell death.
Amiodarone is an antiarrhythmic drug that can cause steatosis. The cationic amphiphilic structure of this drug interferes with mitochondrial activity by uncoupling oxidative phosphorylation at low concentrations and inhibiting respiration at high concentrations.

FURTHER INVESTIGATION OF YOUR COMPOUNDS' MOA ON MITOCHONDRIA:

COMPLEMENTARY TESTS AVAILABLE ON MITOXVIEW®

Drug-induced mitochondrial dysfunction can be further characterized by:

Assessing substrate-dependent oxygen consumption using permeabilized cells and Seahorse technology.
- driven by complex I, complex II activities
- driven by β–oxidation of fatty acids (long/medium chain FA)

Using well-established spectrofluorometric assays
- Transmembrane potential
- mtROS production
- ADP/ATP ratio
- Enzymatic activity of respiratory chain complexes (I to V)

Assessing long-term damage to mitochondrial integrity
- mtDNA depletion

Bibliography
- Begriche K, Massart J, Robin MA, Borgne-Sanchez A, and Fromenty B (2011) J Hepatol
- Dykens JA, Will Y (2007) Drug Discov Today
- Guillouzo A, Corlu A, Aninat C, Glaise D, Morel F, and Guguen-Guillouzo C (2007) Chem Biol Interact
- Porceddu M, Buron N, Roussel C, Labbe G, Fromenty B, and Borgne-Sanchez A (2012) Toxicol Sci
- Porceddu M, Buron N, Rustin P, Fromenty B, and Borgne-Sanchez A. In book: Methods in Pharmacology and Toxicology – Drug-induced liver toxicity, 2018 Eds. M. Chen & Y. Will, Springer LLC, New York (NY, USA).
- Will Y, and Dykens J (2014) Expert Opin Drug Metab Toxicol 10, 1061-1067.

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