LMU LUDWIG-MAXIMILIANS-UNIVERSITÄT MÜNCHEN

Organelles as Tools in Toxicology – In vitro and In vivo Approach

Suhasini Rajan

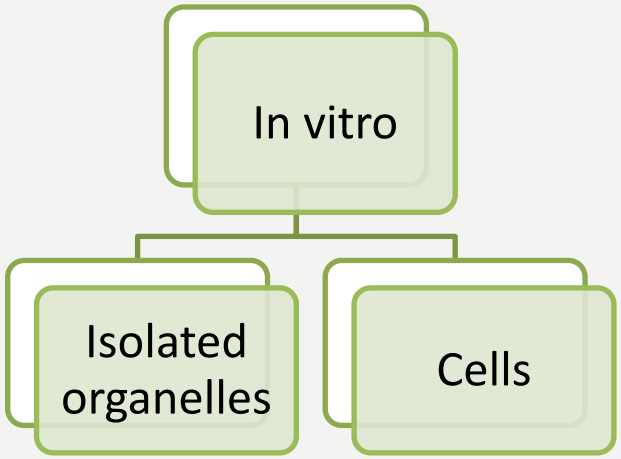
Contents

- Definition of in vitro and in vivo studies
- Role of mitochondria in toxicology
  - Drug toxicity
  - Morphology studies
  - Respiratory assays
  - Detection of reactive oxygen species
- Peroxisome in toxicology
  - Immuno-spin trapping
- Intergrative proteomic approach

Definition of in vitro and in vivo studies

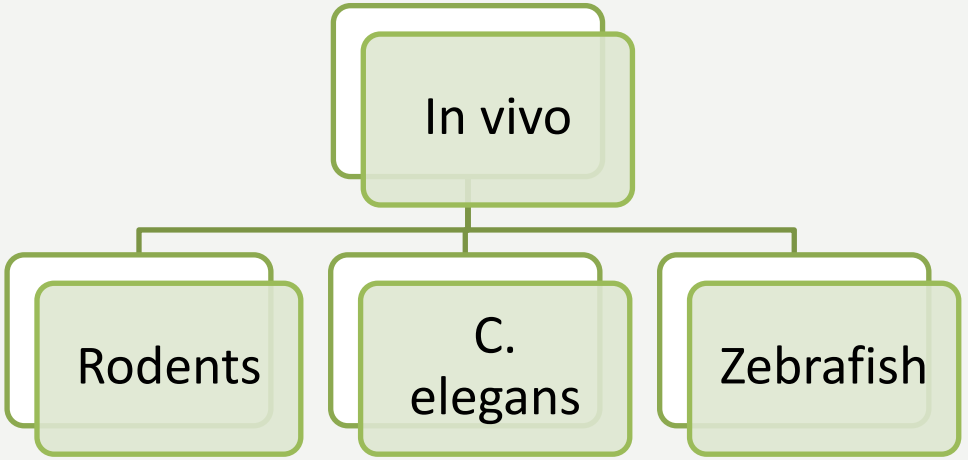
In vitro

- studies are conducted using components of an organism that have been isolated from their usual biological surroundings, such as microorganisms, cells, or biological molecules



In vivo

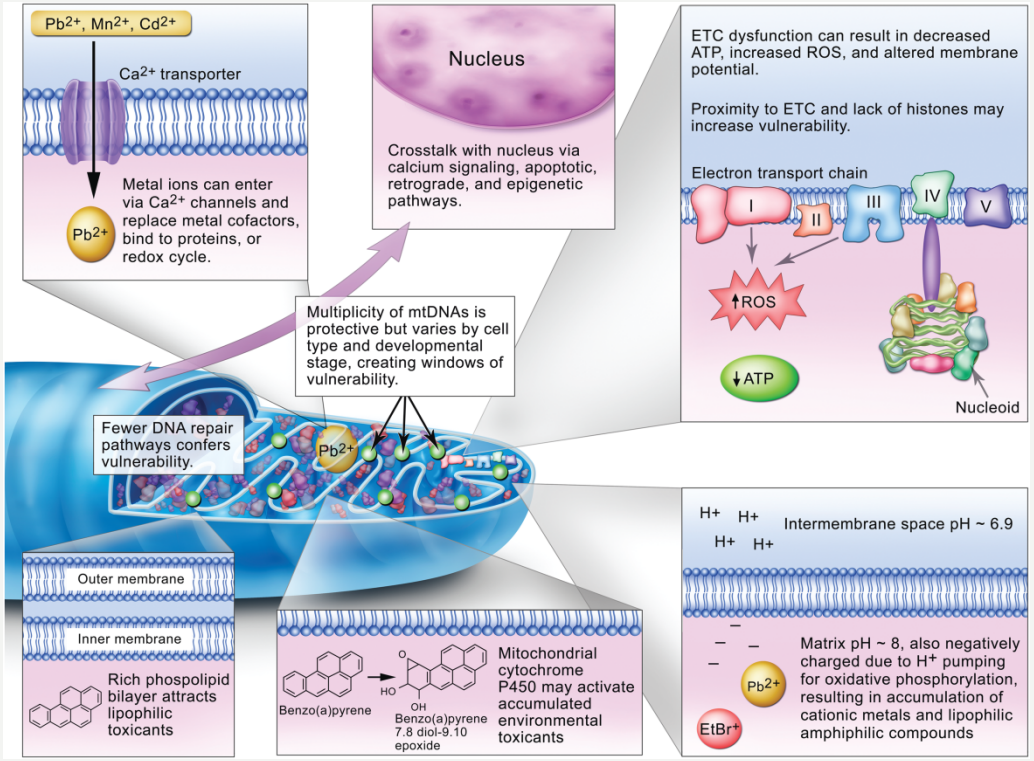
- in which the effects of various biological entities are tested on whole, living organisms or cells, usually animals, including humans, and plants, as opposed to a tissue extract or dead organism



Mitochondria

Functions:

1. Energy production
2. Maintaining homeostasis
3. Balancing oxidation/ reduction reactions
4. Cellular profileration
5. Apoptosis



Meyer, J.N., et al., *Mitochondria as a target of environmental toxicants.* Toxicol Sci, 2013. **134**(1): p. 1-17**.**

Mitochondrial drug toxicity

Table 1
Mitochondrial toxicity of drugs: principle mechanisms and typical examples

Inhibition of the electron transport chain	Amiodarone, anthralin, buprenorphine, flutamide, MPP + , oxmetidine, perhexiline
Uncoupling of oxidative phosphorylation	Amiodarone, bupivacaine, buprenorphine, etidocaine, tacrine
Mitochondrial permeability transition	Salicylate, valproate
Inhibition of mitochondrial fatty acid metabolism	Amiodarone, buprenorphine, female sex hormones, NSAIDs, salicylate, tetracycline, valproate
Oxidation of mitochondrial DNA	Alcohol
Inhibition of mitochondrial DNA synthesis	Nucleoside analogues, e.g. zidovudine, fialuridine

Krähenbühl, S., *Mitochondria: important target for drug toxicity?* Journal of Hepatology, 2001. **34**(2): p. 334-336.

High-throughput Screening

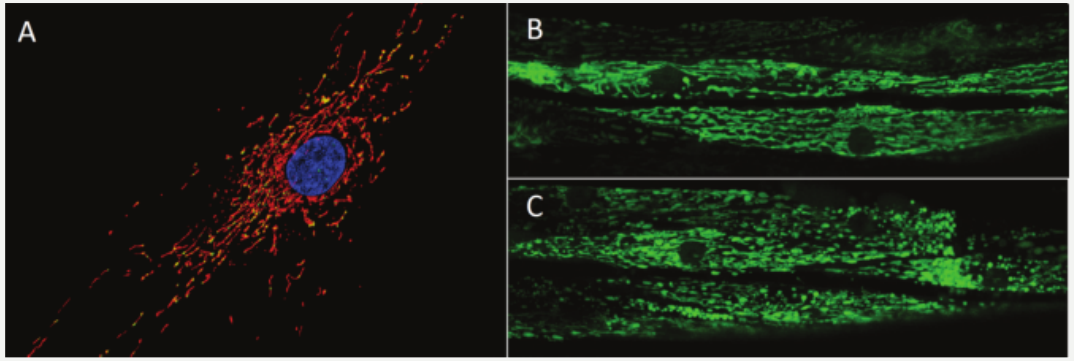
1. Application for large compund libraries
2. Cell culture based approach
3. With Isolated organelles – over or under-predict the effects

Systems for analysing Mitocondrial toxicity:

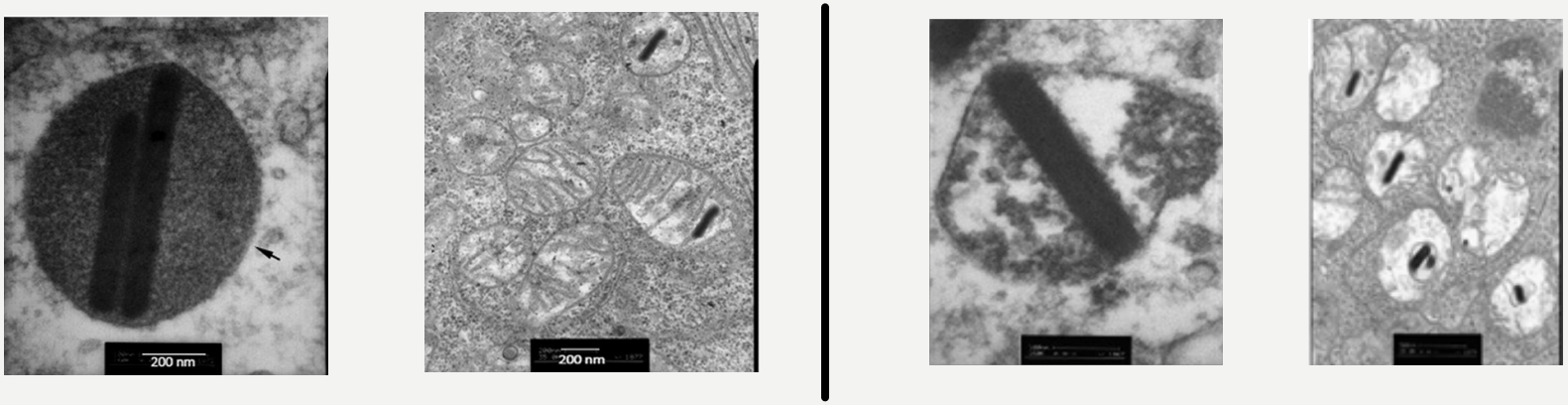
1. Morphology
2. Respirometry
3. Reactive oxygen species (ROS)
4. Membrane potential

Hynes, J., et al., *A high-throughput dual parameter assay for assessing drug-induced mitochondrial dysfunction provides additional predictivity over two established mitochondrial toxicity assays.* Toxicol In Vitro, 2013. **27**(2): p. 560-9.

Morphology



Target and Toxicity of single-walled carbon nanotubules (SWCNT)



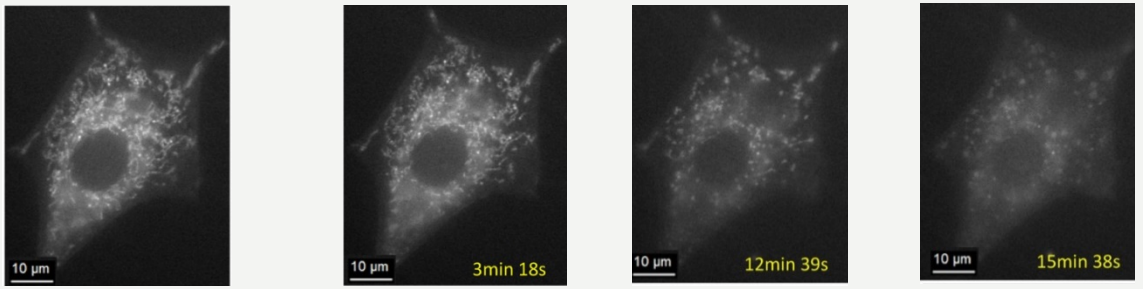
Yang, Z., et al., *Pharmacological and toxicological target organelles and safe use of single-walled carbon nanotubes as drug carriers in treating Alzheimer disease.* Nanomedicine, 2010. **6**(3): p. 427-41**.**

Autofluorescence microscopy

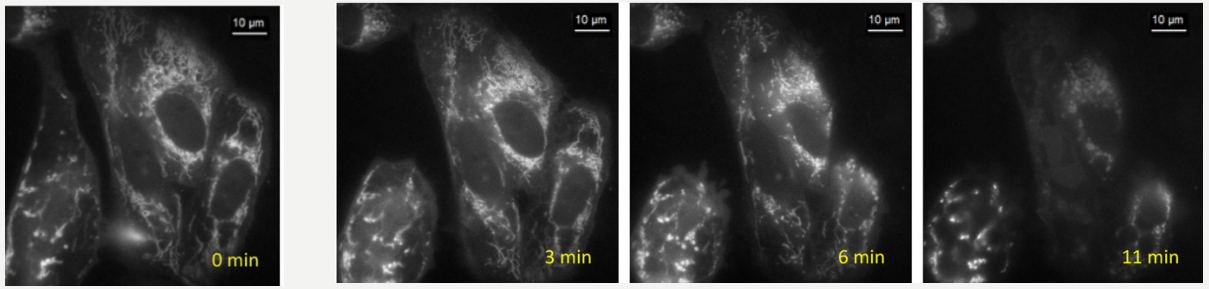
- Continuous non-destructive monitoring
- NADH is a natural fluorophore with excitation and emission spectra between 340 and 450nm
- Quantum yield is higher in mitochondria than in cytosol

Babl/c 3T3cells

+Actinomycin D



Undifferentiated HepaRG cells

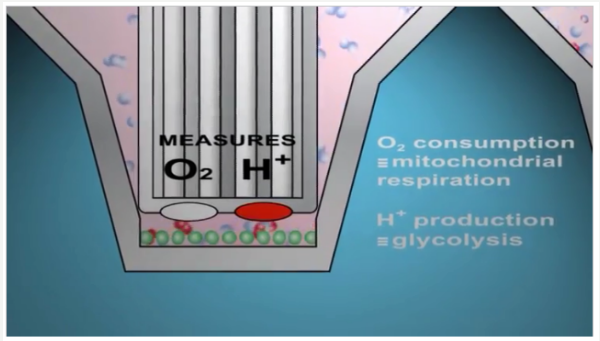


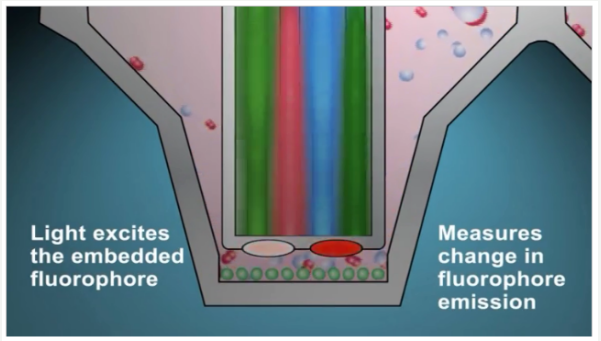
Rodrigues, R.M., et al., *Autofluorescence microscopy: a non-destructive tool to monitor mitochondrial toxicity.* Toxicol Lett, 2011. **206**(3): p. 281-8.

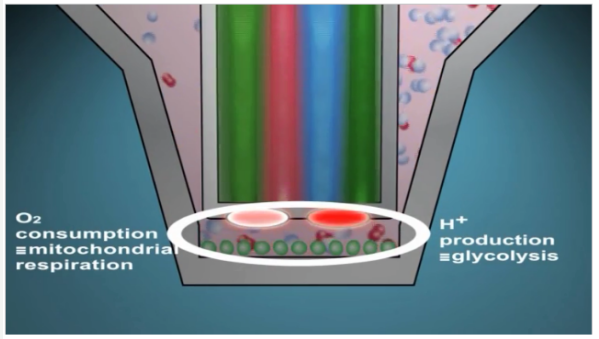
Respirometry

1. ATP content (Luciferin)
2. Oxygen consumption rate
3. Extracellular acidification rate

Extracellular flux analyzer (XF-24, XF-96, Seahorse Biosciences)

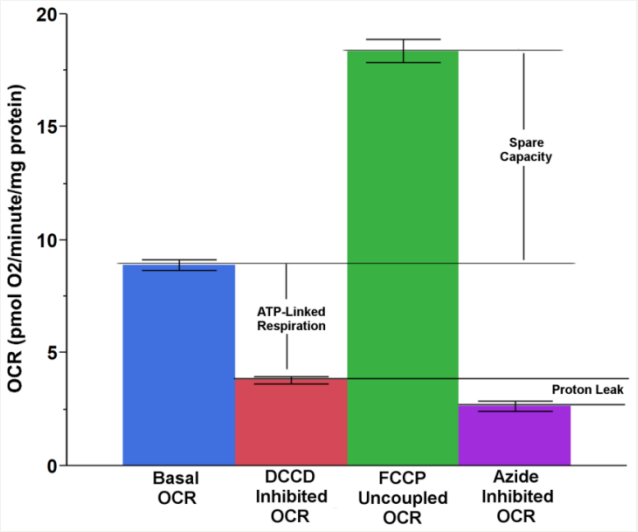




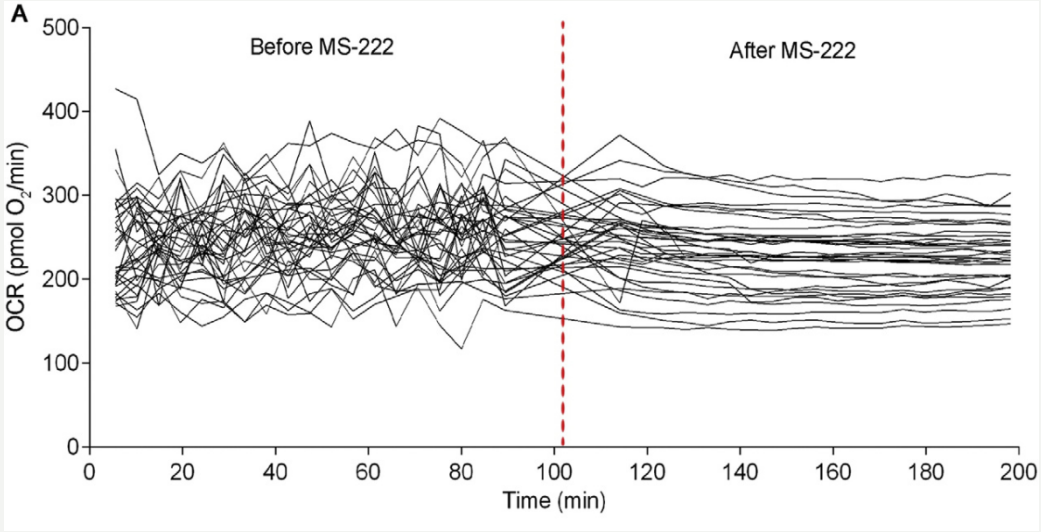


Respirometry- in vivo

Zebrafish



C. elegans



Luz, A.L., et al., *Seahorse Xfe 24 Extracellular Flux Analyzer-Based Analysis of Cellular Respiration in Caenorhabditis elegans.* Curr Protoc Toxicol, 2015. **66**: p. 25 7 1-15.

Raftery, T.D., N. Jayasundara, and R.T. Di Giulio, *A bioenergetics assay for studying the effects of environmental stressors on mitochondrial function in vivo in zebrafish larvae.* Comp Biochem Physiol C Toxicol Pharmacol, 2017. **192**: p. 23-32.

Reactive oxygen species

1. Superoxide anion radicals, hydroxyl radicals, Hydrogen peroxide, singlet oxygen
2. Physiologically, generation and scavenging of ROS is tightly controlled
3. Inefficiencies in electron transport chain
4. Causes Lipid peroxidation, protein oxidation and DNA damage

ROS indicators- dichlorodihydrofluorescein diacetate, hydroethidine, Mito-SOX

Mito-SOX: Mito-hydroethidine is oxidized by superoxide to mito-2-hydroxyethidium and by other ROS to mito-ethidium, exhibit red fluorescence upon interaction with mitochondrial DNA

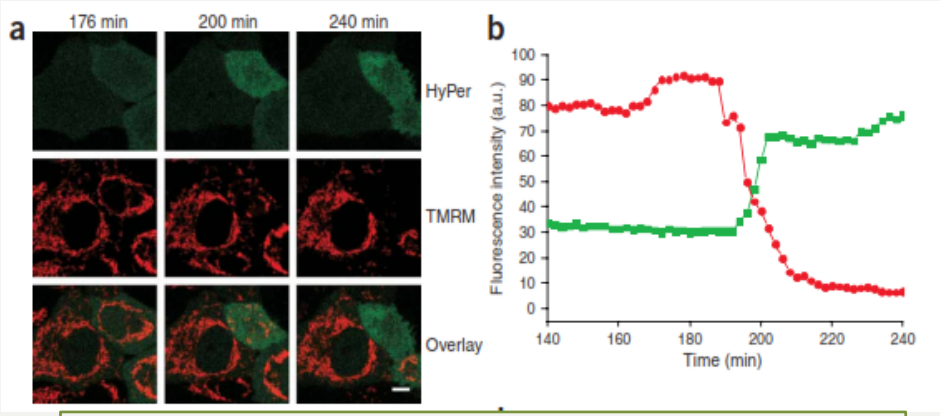
Limitations:

1. Do not react directly with ROS to form fluorescent product
2. Sensitive to light exposure
3. There are also other sources of ROS in a cell

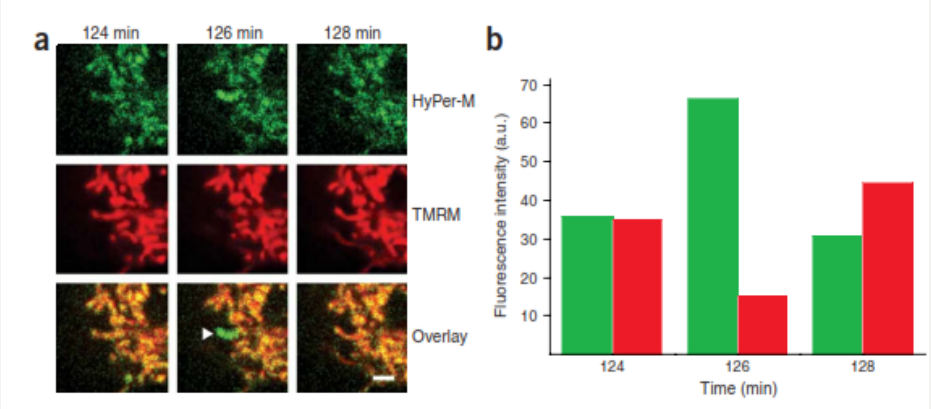
Polster, B.M., et al., *Use of potentiometric fluorophores in the measurement of mitochondrial reactive oxygen species.* Methods Enzymol, 2014. **547**: p. 225-50.

ROS- H_2O_2

Genetically encoded fluorescence



+ Apo2L/TRAIL - apoptogenic protein



HyPer – circularly permuted Yellow fluorescent protein in the regulatory domain of OxyR, a prokaryotic H2O2 sensor

TMRM – Transmembrane potential of Mitochondria

HyPer-M (mitochondria)

Belousov, V.V., et al., *Genetically encoded fluorescent indicator for intracellular hydrogen peroxide.* Nature Methods, 2006. **3**: p. 281.

List of topics

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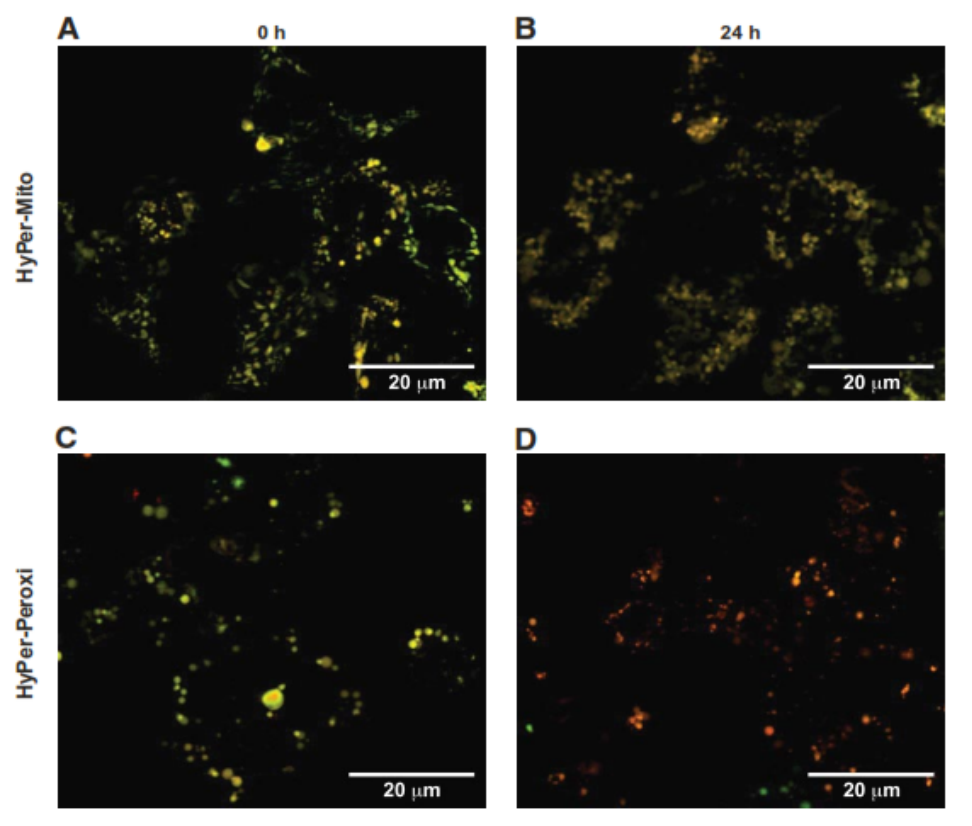
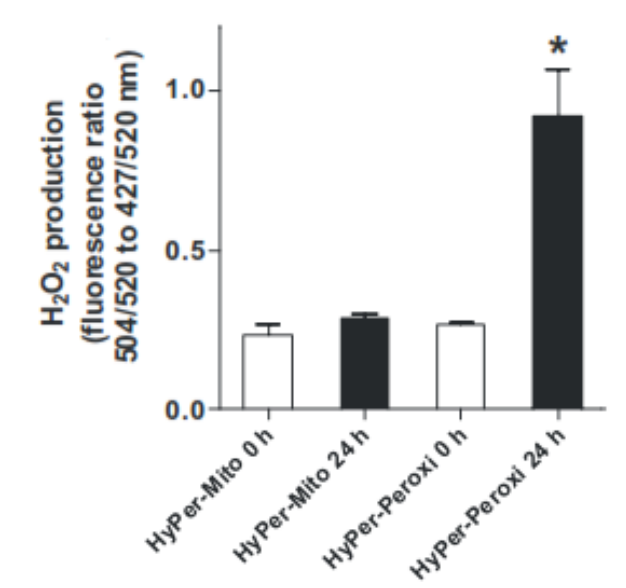
Peroxisome

Fluorescence microscopy of RINm5F insulin-producing cells

+Palmitic acid



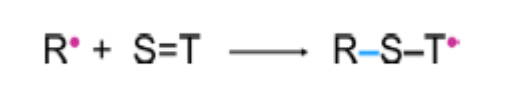


NEFA-induced lipotoxicity in ß-cells in Type II Diabetes

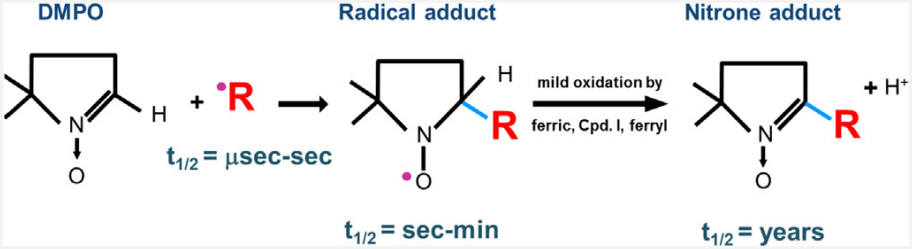
1Elsner, M., W. Gehrmann, and S. Lenzen, *Peroxisome-generated hydrogen peroxide as important mediator of lipotoxicity in insulin-producing cells.* Diabetes, 2011. **60**(1): p. 200-8.

Peroxisome

Spin-trap

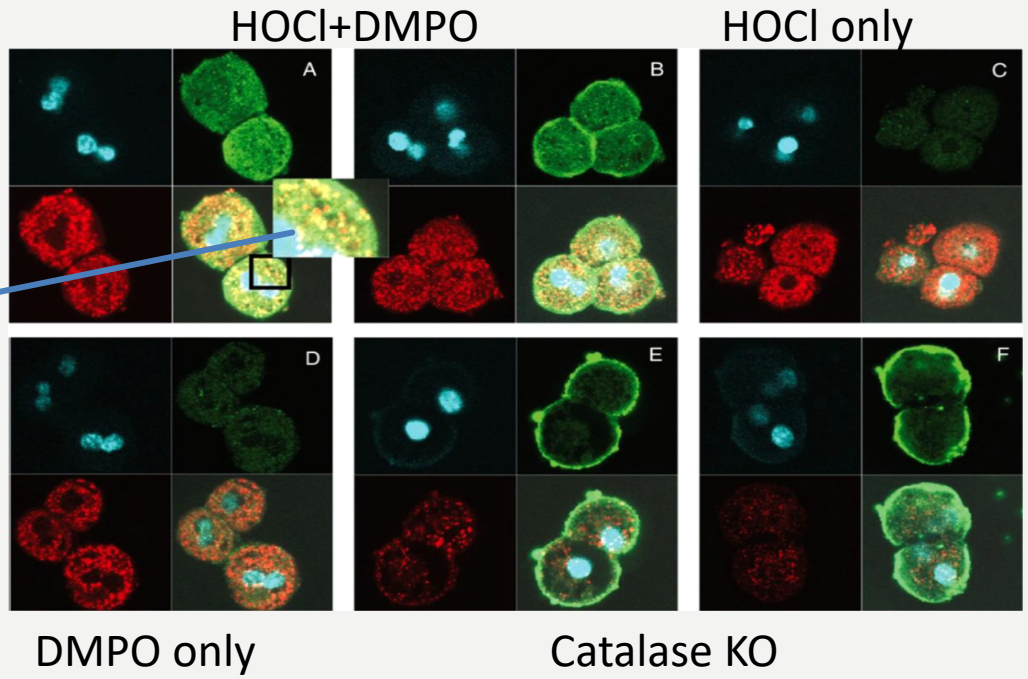


Immuno-spin trapping



Mouse hepatocytes
Red – catalase, green – Protein DMPO adducts

Colocalization of protein DMPO adducts and catalase in peroxisomes



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  - Immuno-spin trapping
- Clinical relevance
  - Intergrative proteomic approach

Clinical relevance

Troglitazone withdrawal from the market

Am J Med. 2003 Mar;114(4):299-306.

Troglitazone-induced liver failure: a case study.

Graham DJ[1], Green L, Senior JR, Nourjah P.

Abstract

BACKGROUND: Troglitazone was removed from the U.S. market because its use was associated with an increased risk of liver failure. We evaluated the clinical features of all cases reported to the Food and Drug Administration and estimated the duration and magnitude of the risk of liver failure associated with continued use of the drug.

Numerous attempts to understand the mechanism of toxicity

Chem Res Toxicol. 2003 Jun;16(6):679-87.

Mechanisms of troglitazone hepatotoxicity.

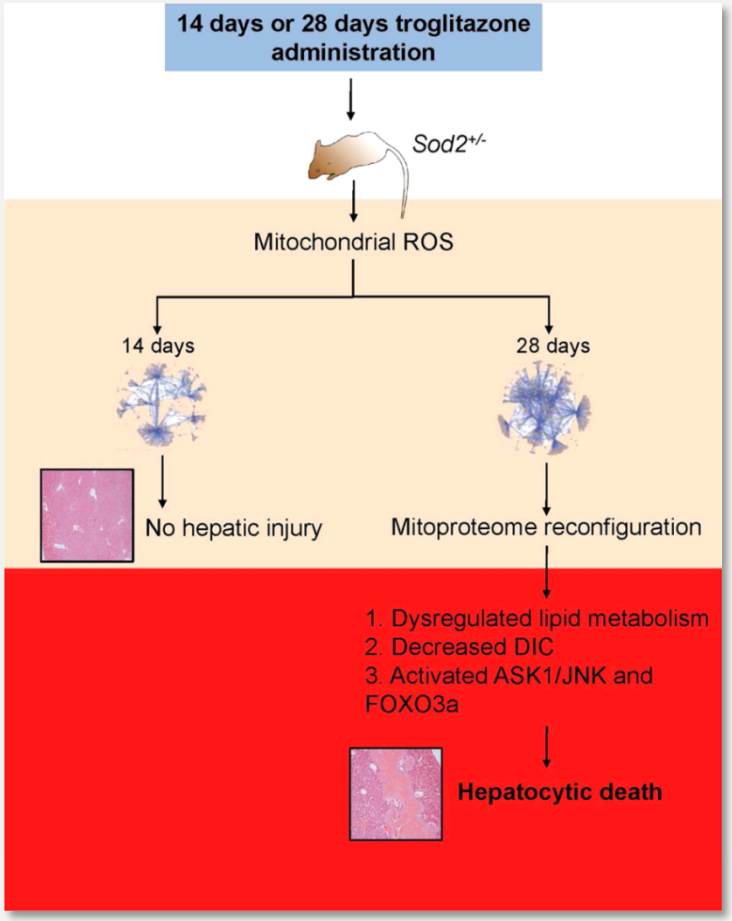
Smith MT[1].

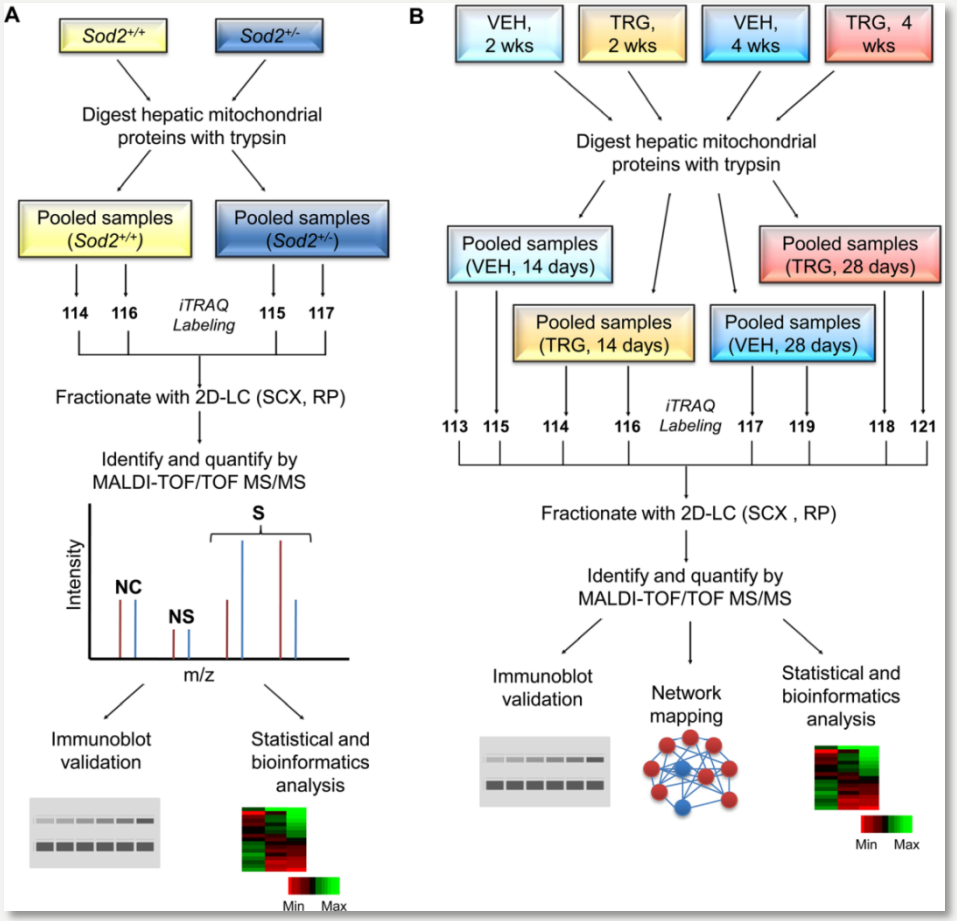
Hepatology. 2005 Feb;41(2):237-46.

Troglitazone and liver injury: in search of answers.

Chojkier M[1].

Clinical relevacne - Integrative proteomics





Lee, Y.H., et al., *Integrative toxicoproteomics implicates impaired mitochondrial glutathione import as an off-target effect of troglitazone.* J Proteome Res, 2013. **12**(6): p. 2933-45.

Thank you