A novel small molecule modulating the mitochondrial NEET (CISD) proteins activity improves inflammation and fibrosis in a diet-induced model of non-alcoholic steatohepatitis

Stéphanie Le Corre[1], Laetitia Lines[1], Diane Sampson[1], Ola Karmi[2], Henri-Baptiste Marjault[2], Rodrigue Rossignol[3], Nivea Dias Amoedo[3], Rachel Nechushtai[2], Raphaël Darteil[1], Jacky Vonderscher[1], Benoît de Chassey[1], and Eric Meldrum[1]
[1]ENYO Pharma, Lyon, France
[2]The Alexander Silberman Life Science Institute, The Hebrew University of Jerusalem, Jerusalem, Israel
[3]Cellomet, Bordeaux, France

INTRODUCTION

Non-alcoholic steatohepatitis (NASH) is a disease characterized by excessive fat accumulation, inflammation, liver cell ballooning and fibrosis in the liver with periportal inflammation having been identified as a marker of disease severity[1]. Here, we present a novel chemistry that regulates the function of 3 mitochondrial proteins called the NEET proteins to target the periportal region of the liver. These proteins play an important role in mitochondrial Fe/ROS homeostasis and the regulation of mitochondrial metabolism.

AIM

Previous literature reports have highlighted the therapeutic importance of NEET proteins in metabolic disease[2,3], so we assessed the possible effects of ENYO's lead NEET protein modulator on the pathology associated with a diet-induced obesity model of NASH in mice and compared those effects with OCA treatment.

METHOD

In vitro experiments

- UV-vis spectroscopy was used to observe the 2Fe-2S cluster stability
- Effects on mitochondrial activity were assessed by evaluating (1) mitochondrial respiration by high-resolution respirometry and (2) the activity of electron transport chain complexes by standardized enzymology assays
- The anti-inflammatory effect has been assessed in a NFKB reporter cell line and in Kupffer cells (IL6 ELISA)

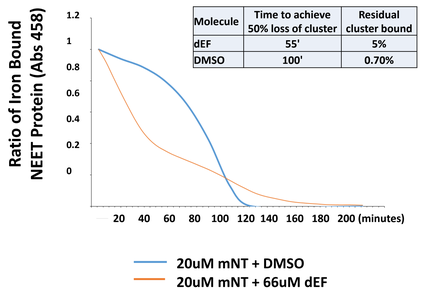
In vivo experiments

- Mice were fed with an AMLN diet (high in fat, fructose and cholesterol) for 30 weeks and then treated by oral administration, twice daily with compounds for 8 weeks. Effects were determined by comparing liver biopsy pathology at baseline and upon study completion. For comparison, a mouse group was also administered OCA daily.

RESULTS

In vitro

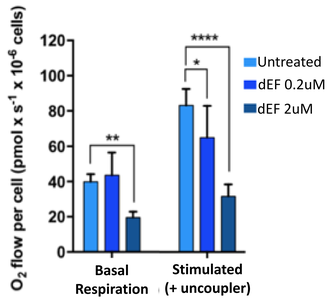
1 Cluster assay stabilization by ENYO's molecule



Molecule	Time to achieve 50% loss of cluster	Residual cluster bound
dEF	55'	5%
DMSO	100'	0.70%

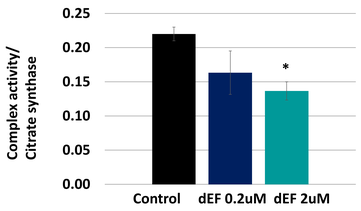
- UV-vis spectroscopy was used to assess the 2Fe-2S cluster stability of NAF-1 or mNT or MiNT in the absence and presence of ENYO's molecule (dEF).
- Our molecule destabilizes the 2Fe-2S cluster of the 3 NEET proteins. As an example we show here the result on mNT.

2 Modulation of Mitochondrial Respiration



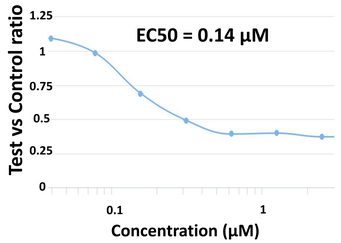
- High-resolution respirometry studies has been performed on A549 cells (cellular oxygen consumption in a closed-chamber system).
- An inhibition of routine respiration and uncoupled maximal respiration (+CCCP) is observed.
- ENYO's molecule inhibits the ability of a cell to enhance oxidative phosphorylation upon stimulation (as induced by metabolic, oxidative or other cellular stress).

3 Inhibition of Electron Transport Chain Complex 1

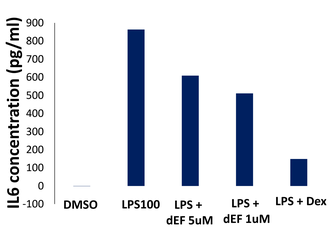


- Rotenone-sensitive NADH-ubiquinone oxido-reductase activity rate is measured spectrophotometrically on cell homogenate and complex I specific function is verified using rotenone, a specific Complex 1 blocker. A significant inhibition of respiratory chain complex 1 activity was measured.

4 ENYO's molecule reduces inflammation *in vitro*



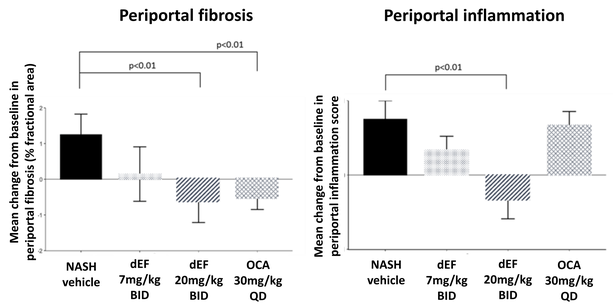
- The NFKB reporter cell line has been induced by TNFα for 6 h
- The signal is detected using a NanoGlo Luciferase Assay System
- ENYO's molecule inhibits NFKB activation by TNFα



- Kupffer cells are induced by LPS for 24h. IL6 production is detected in culture supernatant with an ELISA assay
- Dexamethasone has been used as a positive control
- ENYO's molecule decreases IL6 production in induced Kupffer cells. Of note, the maximum effect is reached at the lowest concentration tested, 1uM.

In vivo

5 Reduction of liver Inflammation and Fibrosis *in vivo*



- Inflammation is graded by number of H&E positive inflammatory foci per field.
- Fibrosis is graded by image analysis and quantification of PSR positive staining in the region 100 µm from the portal tract.
- Therapeutic use of ENYO's molecule resolves periportal inflammation and periportal fibrosis following 8 weeks of treatment in a NASH mouse model.

CONCLUSIONS

ENYO's lead molecule is well tolerated *in vivo* and resolves periportal inflammation and periportal fibrosis in a NASH mouse model.
In the liver, improved pathology is biased to the hepatic periportal zone which is perfused with blood with highest oxygen content[4]. We propose hepatocytes, pro-inflammatory & pro-fibrotic cells in the well-perfused periportal zone are most sensitive to ENYO's molecule by virtue of their bioenergetic predominant oxydative ($OxPhos^+$) status and metabolic inflexibility.

ENYO's lead series offers a novel approach to regulate the activity of a family of proteins of critical importance in mitochondrial biology. It may offer new perspectives in the treatment of NASH and other diseases with an important inflammatory component.

REFERENCES

1- Liver Fibrosis, but no Other Histologic Features, Associates with Long-term Outcomes of Patients With Nonalcoholic Fatty Liver Disease *(Angulo et al 2015, Gastroenterology, 149(2) 389-397)*

2- NAF-1 inhibitor improves diabetic endpoints in db/db mice *(Takahashi et al 2015, J.Pharmacol. Exp. Ther. **352** p338)*

3- NAF-1 haploinsufficiency results in NASH-like pathology *(Shen et al 2017 Cell Reports, **21** p2198*

4- Spatial heterogeneity in the mammalian liver *(Shani Ben-Moshe and Shalev Itzkovitz 2019, Nature reviews)*

CONTACTS

Stéphanie Le Corre: slc@enyopharma.com

Raphael Darteil: rda@enyopharma.com

Eric Meldrum: ecm@enyopharma.com