

Title: *In vitro* study of the selective inhibition of HDAC3 on adipocyte and macrophage phenotype.

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INTRODUCTION and AIM: Histone deacetylases (HDACs) are epigenome modifiers that regulates the phenotype and metabolism of adipocytes and macrophages. HDAC3 genetic inactivation induces metabolic rewiring in white adipose tissue, promoting browning (*Ferrari A et al. (2017) Nat Commun 8, 93*). Additionally, HDAC3 silencing or inhibition reduces cytokine secretion in monocytes and M1 macrophages (*Ghiboub M et al (2020) Frontiers in Immunology*). This project aims to evaluate the effect of LA9498, a novel selective HDAC3 inhibitor, on adipocyte and macrophage phenotype.

METHODS: C3H/10T1/2 adipocytes were treated with vehicle or LA9498 during differentiation, and MS275, a class I HDAC inhibitor, was used as a reference compound. Similarly, RAW264.7 macrophages were treated with vehicle, MS275, or LA9498, in the presence or absence of the proinflammatory cytokine interferon gamma (IFN γ) for 4 hours. Gene expression, protein expression, lipid accumulation, mitochondrial mass and functionality were analyzed at the end of the treatment.

RESULTS: Both compounds upregulated genes involved in adipocyte differentiation, lipid mobilization, fatty acid β -oxidation, browning, and mitochondrial functionality. However, the HDAC3 selective inhibitor LA9498 showed higher potency as compared to MS275. LA9498-treated adipocytes exhibited increased lipid accumulation, enhanced mitochondrial functionality (e.g., ATP production, maximal respiration, spare capacity), and higher expression of proteins belonging to electron transport chain complexes II, III, and IV. When tested in IFN γ -activated macrophages, LA9498 reduced the expression of proinflammatory genes (e.g., *Il1b*, *Tnf*, *Nos2*) and upregulated the mRNA levels of the anti-inflammatory gene *Arg2*. These effects were specific for the HDAC3 selective inhibitor, as MS275 did not affect the expression of these genes.

CONCLUSIONS: LA9498 is a compound that induces browning of adipocytes and promotes anti-inflammatory effects in macrophages. Further studies are warranted to evaluate the effect of this novel compound *in vivo* animal models of obesity and insulin resistance.

Attending the EMBO Workshop in Torremolinos will be a unique chance for me to delve into the latest advancements in adipocyte biology and metabolism. As a first-year PhD student, I look forward to learning from experts in the field and exploring new ideas to further develop my project.