Characterization of EYP001 a novel, potent and selective FXR agonist in *in vitro* 3D human liver models

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INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is a condition defined by excessive fat accumulation (steatosis) in the liver that currently affects 20-40% of the general population. Non-alcoholic steatohepatitis (NASH), the most severe form of NAFLD, is characterized by steatosis, hepatocyte ballooning, inflammation, and fibrosis. It may also lead to complications such as cardiovascular disease, cirrhosis, liver failure or liver cancer. 10-20% of NAFLD patients develop NASH, thus making NASH a significant health burden with currently no approved pharmacological treatments.

The Farnesoid X Receptor (FXR), a nuclear receptor, controls bile acid homeostasis and is a promising target for the treatment of NASH. EYP001, a novel non-steroidal, selective, potent, second generation FXR agonist, is currently in a phase 2 NASH clinical trial.

AIM

Characterize EYP001 in *in vitro* 3D human liver models in comparison to OCA and other FXR agonists currently in clinical development.

METHODS

- **3D InSight™ human liver microtissues (InSphero)**

Cytotoxicity in this model was assessed upon exposure to EYP001, OCA (both from 0.03 to 30 µM) and the two OCA derivatives Glyco-OCA (G-OCA) and Tauro-OCA (T-OCA), both from 0.01 to 10 µM, by LDH release in the supernatant and intracellular ATP content for up to 7 days. Bile acid (BA) secretion profiles (glycocholic acid = GCA, and glycochenodeoxycholic acid = GCDCA) were analyzed at day 2 by LC/MS for these compounds and four other FXR agonists currently in clinical development in NASH (labelled as compounds A, B, C and D). Three independent experiments were carried out for all 3D InSight human liver microtissue data.

- **3D Bioprinted ExVive™ human liver tissues (Organovo)**

EYP001 protective effects against NASH (induction by treatment with glucose, fructose and palmitate) were assessed in 3D bioprinted ExVive™ human liver tissues following 21 days of daily treatments. PLIN2 and αSMA positive areas were digitally quantified via ImageJ. For each experimental condition, 4 microtissues were treated and prepared for histology.

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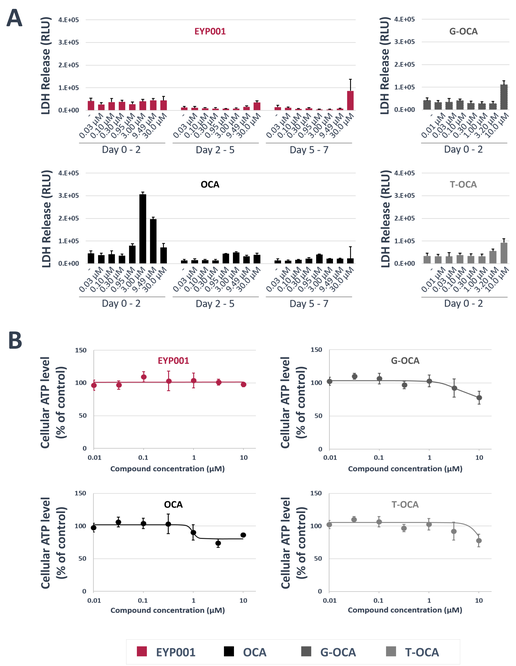
RESULTS

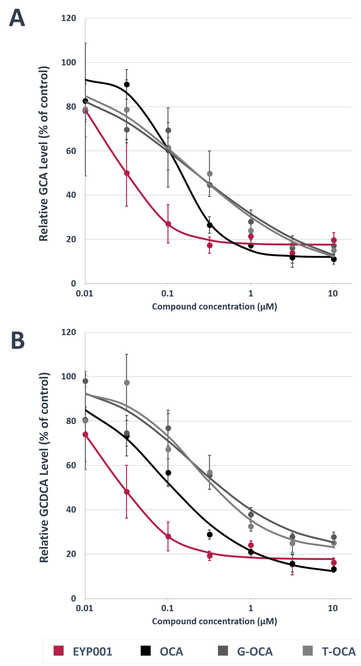
3D InSight™ human liver microtissues

EYP001, contrary to OCA, is not cytotoxic at effective doses



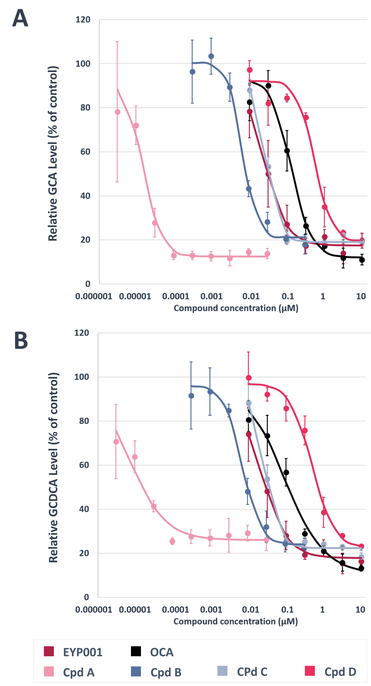
EYP001 was found not cytotoxic to 3D InSight™ human liver microtissues throughout the experiment (only a mild cytotoxicity was measured at 30 µM at day 7 in LDH release). Unlike EYP001, OCA showed strong cytotoxicity at 3 and 10 µM at day 2 in LDH release (**A**), and in some intracellular ATP content too (**B**). Some mild cytotoxicity was observed at the highest dose (10 µM) for G-OCA and T-OCA at day 2, both in LDH release and ATP content.

EYP001, OCA and its derivatives decrease BA secretion in a dose-dependent manner



All tested compounds (EYP001, OCA and its 2 derivatives G-OCA and T-OCA) showed a dose-dependent decrease of GCA (**A**) and GCDCA (**B**) secretion within days 0-2. EC50 were as follow : EYP001 22 and 19 nM, OCA 128 and 87 nM, G-OCA 180 and 201 nM, and T-OCA 184 and 231 nM, for GCA and GCDCA respectively.

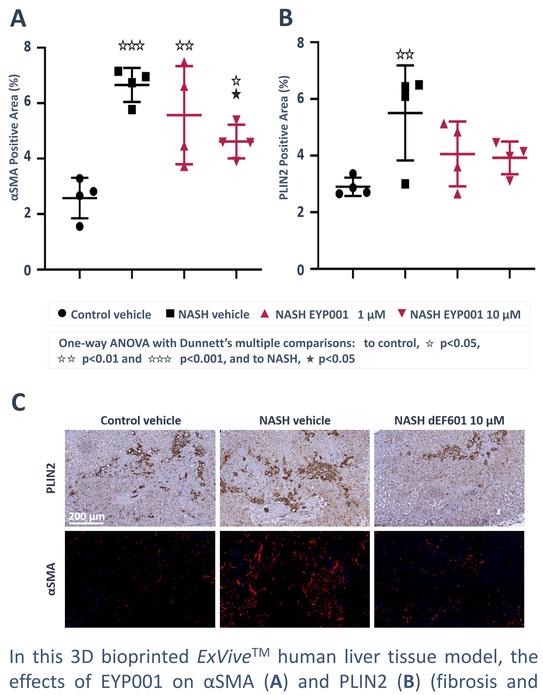
All 6 FXR agonists in development against NASH decrease bile acid secretion



All 6 tested compounds (EYP001, OCA and 4 undisclosed FXR agonists currently in clinical development against NASH) showed a dose-dependent decrease of secretion of GCA (**A**) and GCDCA (**B**). The EC50 of the most potent compound (Cpd A) were 15 and 7 pM for GCA and GCDCA respectively, and 500 nM for the less potent (Cpd D) (for both BA).

3D ExVive™ human liver tissues

EYP001 reduces αSMA (fibrosis marker) and PLIN2 (steatosis marker) in a dose dependent manner



In this 3D bioprinted *ExVive*™ human liver tissue model, the effects of EYP001 on αSMA (**A**) and PLIN2 (**B**) (fibrosis and steatosis markers respectively) were digitally quantified from histological slides (**C,** representative tissue sections). EYP001 reduced PLIN2 positive area in a dose dependent manner compared to the NASH condition. This result suggests a decreased presence of lipid droplets in both EYP001 conditions. Also, EYP001 significantly reduced αSMA positive area at both concentrations tested, suggesting less activation of stellate cells.

CONCLUSIONS

The non-steroidal FXR agonist EYP001 was shown to be not cytotoxic in 3D human liver tissues *in vitro* and to be more potent than OCA on reduction of bile acid secretion. EYP001 reduced PLIN2 and αSMA, two parameters linked to lipid accumulation and fibrosis respectively, strongly suggesting an improvement of NASH parameters in this 3D *in vitro* human liver model. Based on these preclinical efficacy findings, and safety, tolerability, pharmacokinetics and pharmacodynamics data obtained in Phase 1 clinical trials, EYP001 has now entered a Phase 2 clinical trial examining benefit in NASH patients.

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