ENANTA Pharmaceuticals

#1568

EDP-305, A Novel and Highly Potent Farnesoid X Receptor Agonist, Exerts Favorable Effects on Lipid Metabolism *In Vitro*

Yang Li, Mary Dinh Le Chau, Guoqiang Wang, Yat Sun Or, and Lijuan Jiang
Enanta Pharmaceuticals, Inc. Watertown, MA, USA

To request a pdf of this poster, email **ljiang@enanta.com**

Introduction

Nonalcoholic steatohepatitis (NASH), characterized by steatosis, lobular inflammation, perisinusoidal fibrosis and hepatocyte ballooning, develops in a significant proportion of patients with non-alcoholic fatty liver disease (NAFLD). NAFLD currently affects 20-40% of the general population, with 10-20% patients developing NASH. NASH is therefore becoming a major health issue in close association with obesity and diabetes.[1]

The farnesoid X receptor (FXR) is a member of the nuclear receptor superfamily that was originally identified as the physiological receptor for bile acids. FXR is mainly expressed in liver, intestine, kidney, and, to a lesser extent, adipose tissues. Activation of FXR regulates multiple metabolic pathways including bile acid, lipid and glucose homeostasis, as well as immune responses. FXR activation inhibits hepatic *de novo* lipogenesis, increases insulin sensitivity and protects hepatocytes against bile acid-induced cytotoxicity[2]. Obeticholic acid (OCA), a 6a-ethyl derivative of chenodeoxycholic acid (CDCA), is a first-in-class selective FXR agonist. Recent results from the FLINT clinical trial testing the efficacy of OCA in NASH, demonstrated improvement among NASH patients. One of the major side effects, however, following OCA treatment was dyslipidemia, as indicated by elevated levels of LDL-C and reduced levels of HDL-C[3]. Herein, the effects of EDP-305, a novel and highly potent farnesoid X receptor (FXR) agonist, on lipid metabolism were evaluated side-by-side with OCA *in vitro*.

Methods

Cell culture and treatments

The human hepatoma cell line, Huh7.5, was routinely maintained in growth media containing DMEM supplemented with 10% FBS, 1mM Sodium Pyruvate, 1mM non-essential amino acid and 1% penicillin-streptomycin. Cells were treated with EDP-305 or OCA at various concentrations.

Triglyceride (TG) synthesis and LDL uptake

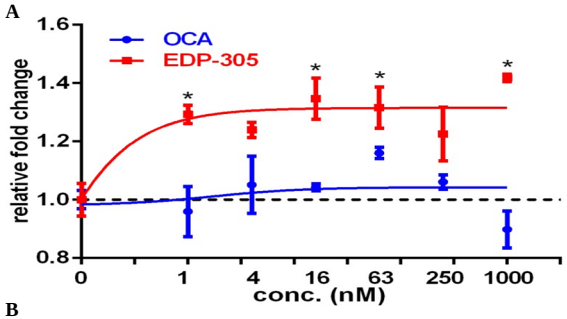
Cells were pre-treated with EDP-305 or OCA for 18hrs at indicated concentrations. To study genes involved in TG synthesis, cells were then co-treated with the LXR agonist TO901317 (100nM) for an additional 6hrs. Gene expression was determined by RT-PCR. For TG accumulation, cells were first pre-treated with chemicals for 18hrs and then co-treated with palmitate (100uM) for an additional 4hrs. TGs were quantified using the Wako diagnostic kit. In the LDL-c uptake assay, Huh7.5 cells were treated with EDP-305 or OCA for 8hrs. Cells were then treated with BODIPY-LDL-C (Molecular Probes) for 0.5hrs. Fluorescence was quantified at 505/535 nM (Ex/Em).

References

1. Adorini, L et al. 2012. Farnesoid X receptor targeting to treat nonalcoholic steatohepatitis. *Drug Discov Today*. **17**: 988-97.
2. Schaap, F et al. 2014. Bile acid receptors as targets for drug development. *Nat Rev Gastroenterol Hepatol*. **11**: 55-67.
3. NASH Clinical Research Network. 2015. Farnesoid X nuclear receptor ligand obeticholic acid for non-alcoholic steatohepatitis (FLINT): a multicenter, randomized, placebo-controlled trial. *Lancet*. **385**:956-65.

Results

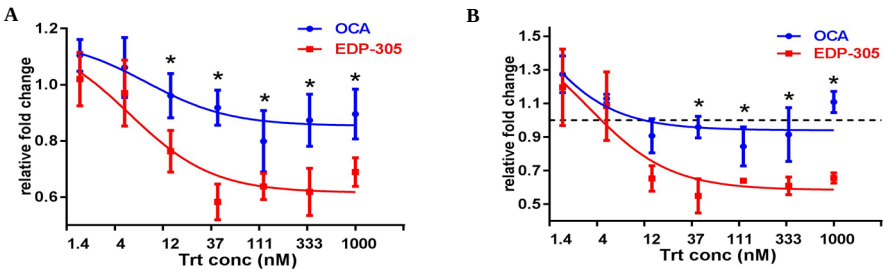
Fig 1. EDP-305 dose-dependently increases LDL receptor (LDLr) expression and LDL uptake. A. At concentrations close to their respective EC_{50} values (16nM and 1μM), EDP-305 significantly increased LDLr expression by 30%, while OCA decreased LDLr expression by 10%. **B**. At the concentration of 0.5 μM, EDP-305 treatment resulted in a 26% elevation in LDL uptake by hepatocytes, whereas OCA had no significant effect on LDL uptake.



** p<0.05 between OCA and EDP-305.*

** p<0.05 between EDP-305 and control.*

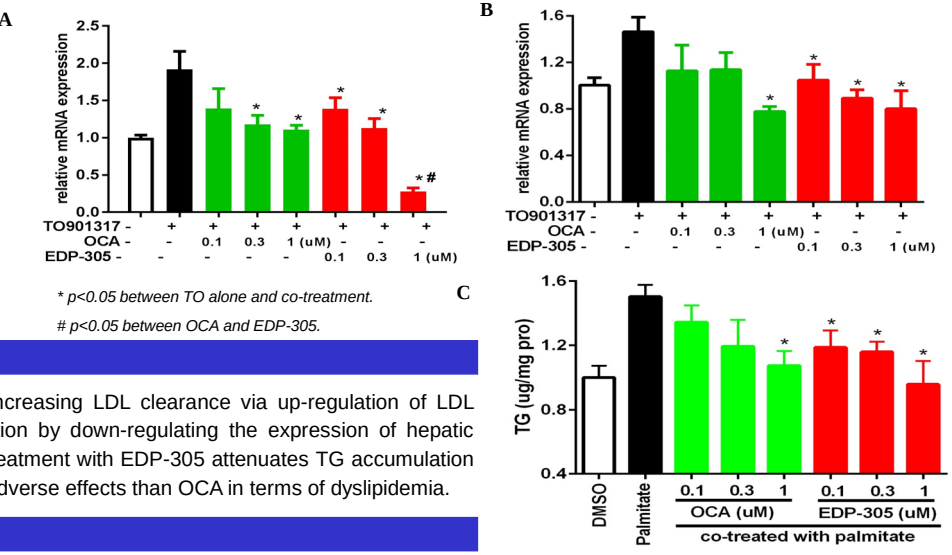
Fig 2. EDP-305 dose-dependently decreases APOC3 and HL expression. A. EDP-305 decreased expression of apolipoprotein C3 (apoC3), an inhibitor of lipoprotein lipase, and exhibited better potency than OCA in the concentration range of 12 to 1000 nM. **B**. EDP-305 dose-dependently decreased hepatic lipase (HL) expression, while OCA had no significant effect.



** p<0.05 between OCA and EDP-305.*

** p<0.05 between OCA and EDP-305.*

Fig 3. EDP-305 significantly reduces the expression of genes involved in triglyceride (TG) synthesis, and TG accumulation. EDP-305 significantly decreased the expression of sterol regulatory element-binding protein 1c (**A**) and stearoyl CoA desaturase 1 (**B**) by 50% and 60%, respectively. **C**. In palmitate-treated cells, treatment with EDP-305 dose dependently decreased TG accumulation, comparable to basal levels found in the hepatocytes.



** p<0.05 between TO alone and co-treatment.*

 # p<0.05 between OCA and EDP-305.

** p<0.05 between palmitate alone and co-treatment.*

Conclusions

EDP-305 positively affects the lipid profile *in vitro*, by potentially increasing LDL clearance via up-regulation of LDL receptor. Moreover, EDP-305 can potentially reduce HDL degradation by down-regulating the expression of hepatic lipase. In addition to its positive effects on lipoprotein metabolism, treatment with EDP-305 attenuates TG accumulation in palmitate-treated cells. Thus, EDP-305 may potentially have less adverse effects than OCA in terms of dyslipidemia.

Acknowledgements

We thank Ruichao Shen for preparing all the compounds used in these studies. We also thank Jun Zhang and Kristen Sagliani for their advice on this poster.