ENANTA Pharmaceuticals

EDP-305, a potent and selective farnesoid X receptor agonist, exhibits anti-fibrotic effect *in vitro*

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

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

Background

Nonalcoholic steatohepatitis (NASH) is becoming a major global health burden, with increasing prevalence and incidence worldwide. There is no approved therapy for the treatment of NASH. The farnesoid X receptor (FXR) has emerged as an important therapeutic target for the treatment of NASH [1]. EDP-305, a novel FXR agonist, was designed to meet this unmet medical need.

Methods

EDP-305 was tested side-by-side with obeticholic acid (OCA) for potency (EC_{50}) and efficacy using both a chimeric FXR reporter assay in Chinese hamster ovary (CHO) cells and a full length FXR luciferase reporter assay in human embryonic kidney (HEK) cells [2,3]. The ability of EDP-305 and OCA to regulate FXR downstream gene expression of small heterodimer partner (SHP), cholesterol 7 alpha-hydroxylase (CYP7A1) and bile salt export pump (BSEP) was tested in the human Huh7.5 hepatocyte cell line. The *in vivo* activity of EDP-305 and OCA was determined in C57BL/6 mice treated by oral gavage with EDP-305 or OCA at the indicated doses for five days.

Human primary hepatic stellate cells (HSC) were treated with 5 ng/ml transforming growth factor beta (TGFβ) alone or with a combination of 5 ng/ml of TGFβ with 500 nM of EDP-305 or OCA to assess the effects of EDP-305 on liver fibrosis. Key inflammatory and fibrotic genes were analyzed by RT-PCR.

Results

EDP-305 is a potent FXR agonist with an EC_{50} value of 8 nM in a full-length FXR reporter assay using HEK cells, which is a16-fold greater potency than OCA (EC_{50}: 130 nM). In addition, EDP-305 does not activate other nuclear receptors and is selective for FXR. Both EDP-305 and OCA showed dose-dependent increases in SHP and BSEP gene expression *in vitro*. At 12 nM, which is close to the EC_{50} of EDP-305 in the HEK cell reporter assay, EDP-305 induced SHP and BSEP mRNA expression by 5-fold and 18-fold, respectively, compared to minimal induction by OCA. EDP-305 reduced CYP7A1 mRNA expression down to approximately 5%, while 40% CYP7A1 mRNA remained with OCA treatment at 12 nM.

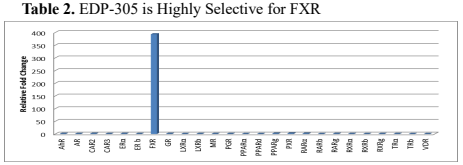
Consistent with the *in vitro* activation of FXR signaling, EDP-305 had similar effects *in vivo,* such that it induced a dose-dependent increase in the expression of target genes: SHP and fibroblast growth factor 15 (FGF15), in the mouse ileum. Moreover, EDP-305 treatment exhibited a dose-dependent increase in SHP and BSEP mRNA, and a dose-dependent reduction in CYP7A1 mRNA in the mouse liver.

In comparison to OCA, EDP-305 significantly displayed more potent anti-fibrotic effects when compared to OCA. For example, when compared to OCA, EDP-305 significantly ($p<0.05$) decreased expression of α-smooth muscle actin (α-SMA) by 68%, collagen type 1 α2 (COL1A2) by 42%, and collagen type 3 α1 (COL3A1) by 57%. Moreover, when compared to OCA, EDP-305 even further decreased expression ($p<0.01$) of metallopeptidase inhibitor 1 (TIMP1) by 80% and metallopeptidase inhibitor 2 (TIMP2) by 65%, which are critical genes involved in the progression of liver fibrosis.

Table 1. Potency and efficacy of EDP-305 and its metabolites in a full length FXR activation assay in HEK cells

Compound	Full length FXR activation (HEK)		
	N	EC50 (μM)	Efficacy (%)
CDCA	10	8.358 ± 1.392	100% ± 2%
OCA	10	0.130 ± 0.039	150% ± 21%
EDP-305	10	0.008 ± 0.003	152% ± 19%
2571 (EDP-305 Metabolite 1)	4	0.011 ± 0.012	149% ± 14%
2572 (EDP-305 Metabolite 2)	4	0.016 ± 0.019	151% ± 19%

Table 2. EDP-305 is Highly Selective for FXR



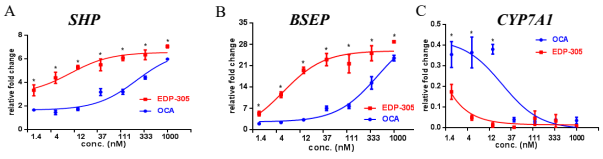
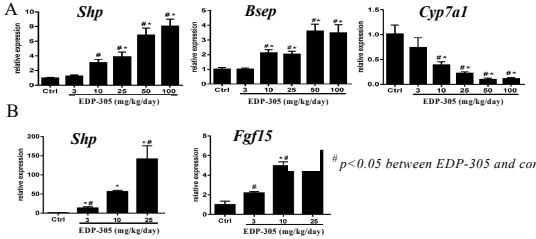


Figure 1. EDP-305 increases SHP, BSEP and CYP7A1 gene expression *in vitro*. HuH7.5 cells were seeded in tissue culture plates overnight in serum-reduced media (1% FBS). Cells were treated with EDP-305 or OCA for 10 hours (n=3). DMSO (0.2% v:v) served as a control. The highest treatment concentration for both EDP-305 and OCA were 1 uM with a series of 1:3 dilutions. (A) SHP; (B) BSEP; (C) CYP7A1. * *p<0.05 between EDP-305 and OCA.*



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

Figure 2. EDP-305 regulates gene expression *in vivo*. C57/Bl6J mice, aged 8-10 weeks old, were treated with EDP-305. EDP-305 was administered via oral gavage. Gene expression analysis in liver (A) and intestine (B) were determined by real-time PCR. 18S ribosomal RNA (18S rRNA) was used as a house-keeping gene control.

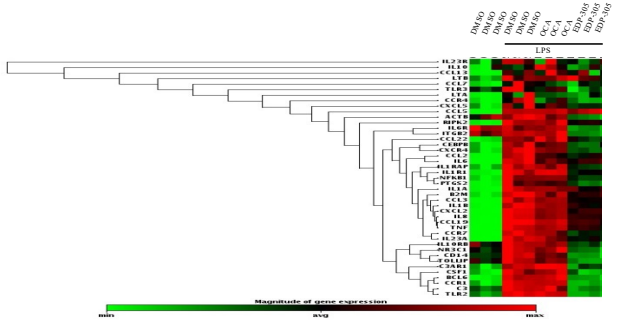


Figure 3. Heat map showing the effects of EDP-305 and OCA on expression of genes involved in inflammation. Relative expression of genes, normalized to control, was calculated from delta CT values. EDP-305 down-regulated inflammatory response genes.

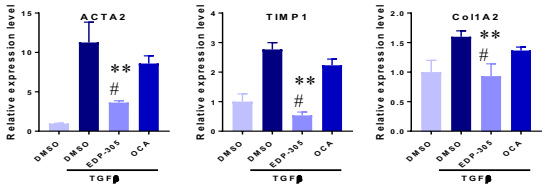


Figure 4. Activation of FXR signaling with EDP-305 significantly inhibited expression of key fibrosis genes *in vitro*. HSCs cells were treated with TGF (5 ng/ml) alone or in combination with OCA (0.5 μM) or EDP-305 (0.5 μM) for 18 hours (n=3 for each treatment).
# P<0.05 compared to LPS; ** P<0.05 compared to OCA.

Conclusions

EDP-305 is a highly potent and selective FXR agonist, and demonstrated greater efficacy than OCA in the regulation of FXR target genes in bile acid metabolism both *in vitro* and *in vivo*. EDP-305 is more potent than OCA in reducing expression of key fibrotic genes *in vitro*, thus holding the potential to mitigate the fibrotic responses associated with NASH.

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 the poster.

References

1. Adorini, L et al. 2012. Farnesoid X receptor targeting to treat nonalcoholic steatohepatitis. *Drug Discov Today.* **17**: 988-97
2. Molecular Pharmacology, 78:617–630, 2010
3. Chimeric FXR assay: http://indigobiosciences.com/products/fxr-nr1h4/