ENANTA Pharmaceuticals

#1540

EDP-305, A Novel and Selective Farnesoid X Receptor Agonist, Exhibits Excellent Potency and Efficacy *In Vitro and In Vivo*

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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

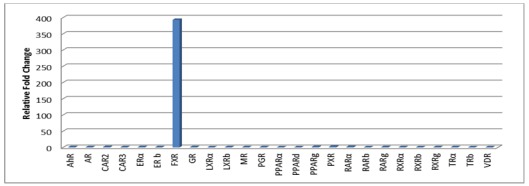
EDP-305 was tested side-by-side with obeticholic acid (OCA) for potency (EC_{50}) and efficacy using both a chimeric FXR reporter assay in Chinese hamster ovary (CHO) cells and a full length FXR luciferase reporter assay in human embryonic kidney (HEK) cells [2,3]. Activation of TGR5 was assessed by measuring the cellular level of cyclic adenosine monophosphate (cAMP) using a competitive immunoassay that quantitates based on enzyme fragment complementation. 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.

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). EDP-305 has minimal activity against TGR5 while OCA is active against TGR5. 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.

Table 1. EDP-305 is Highly Selective for FXR



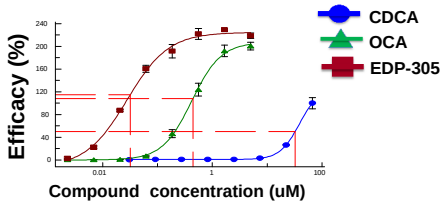


Figure 1. Activation of FXR by EDP-305. Chimeric FXR and luciferase reporter constructs were transfected into CHO cells. Luciferase signal was measured after 22 hours of treatment with CDCA (chenodeoxycholic acid), OCA, or EDP-305.

Table 2. Potency and efficacy of EDP-305 and its metabolites in a chimeric FXR activation assay in CHO cells

Compound	Chimeric FXR activation (CHO)		
	N	EC50 (μM)	Efficacy (%)
CDCA	198	27.972 ± 0.915	100% ± 9%
OCA	198	0.569 ± 0.096	213% ± 39%
EDP-305	70	0.034 ± 0.008	223% ± 42%
2571 (EDP-305 Metabolite 1)	6	0.024 ± 0.011	195% ± 37%
2572 (EDP-305 Metabolite 2)	4	0.134 ± 0.037	176% ± 28%

Table 3. 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 4. Potency and efficacy of EDP-305 and its metabolites in a TGR5 activation assay in CHO cells

Compound	TGR5 activation (CHO)		
	N	EC50 (μM)	Efficacy (%)
LCA (lithocholic acid)	56	0.129 ± 0.043	100% ± 9%
OCA	56	0.381 ± 0.102	72% ± 11%
EDP-305	14	>15	NA
2571 (EDP-305 Metabolite 1)	6	>15	NA
2572 (EDP-305 Metabolite 2)	4	>15	NA

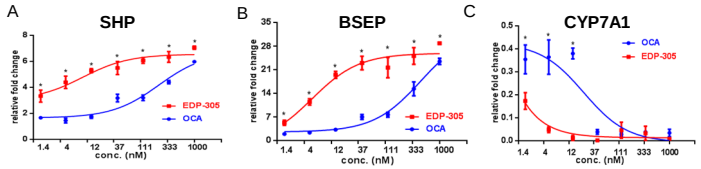
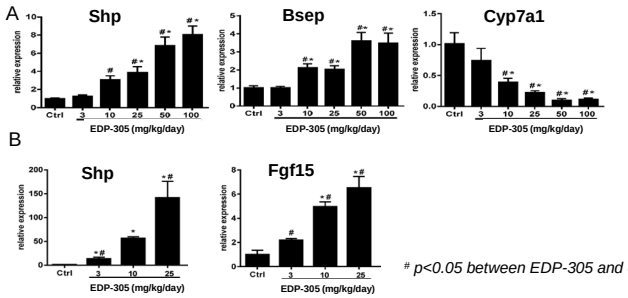


Figure 2. EDP-305 increases SHP, BSEP and CYP7A1 gene expression *in vitro*. HuH7.5 cells were seeded in tissue culture plates overnight in serum-reduced media (1% FBS). Cells were treated with EDP-305 or OCA for 10 hours (n=3). DMSO (0.2% v:v) served as a control. The highest treatment concentration 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 3. 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.

Conclusions

EDP-305 is a highly potent and specific FXR agonist with minimal activity against TGR5. In contrast, OCA showed dual-agonist activity against both FXR and TGR5. EDP-305 demonstrated greater efficacy than OCA in the regulation of FXR target genes in bile acid metabolism both *in vitro* and *in vivo*.

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

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