A novel FXR agonist EDP-297 exerts anti-inflammatory and hepatoprotective effects in human liver 3D microtissues and in rodent models of liver injury and NASH

Mary D.L. Chau, Manuel Roqueta-Rivera, Guoqiang Wang, Kelsey Garlick, Bindu Ramachandran, Kevin Mongeon, Nadya Bryan, Yat Sun Or, and Bryan Goodwin

Enanta Pharmaceuticals, Inc., Watertown, MA, USA

ENANTA Pharmaceuticals

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BACKGROUND AND AIM

The farnesoid X receptor (FXR) plays a central role in regulating bile acid and lipid homeostasis. In both preclinical and clinical settings, FXR agonists decrease liver steatosis and exert anti-inflammatory and anti-fibrotic effects, making FXR an attractive target for the management of non-alcoholic steatohepatitis (NASH). Here we report the identification of a novel, potent FXR agonist EDP-297. We evaluated the pharmacologic activity of EDP-297 in multiple *in vitro* assays and further characterized it in a human liver microtissue system and in rodent models of NASH and liver injury.

METHODS

In Vitro. Experiments using the 3D InSight Human Liver Microtissue NASH model were performed by InSphero (Schlieren, Switzerland). RNA sequencing of microtissues treated with 1 μM EDP-297 or vehicle for 10 days was performed for gene expression analysis.

In vivo. Male Sprague-Dawley rats that underwent sham or bile duct ligation (BDL) procedure (Charles River Laboratories, Wilmington, MA) were treated with 0.3 mg/kg or 1 mg/kg EDP-297 or vehicle, starting on Day 2 post surgery, for 5 days. Gene expression analysis was measured by qPCR. Histological analysis was performed by Charles River Laboratories. Male, biopsy-confirmed, AMLN diet-induced obese NASH (DIO-NASH) mice (Gubra, Hørsholm, Denmark) were treated with vehicle, 0.3 or 1 mg/kg EDP-297 for 12 weeks prior to sacrifice. Multiple markers of hepatic function and liver histology were evaluated. All plasma biochemistry and cytokine levels were measured on the IDEXX Catalyst One analyzer and by Meso Scale Diagnostics assay (Rockville, MD), respectively.

RESULTS

EDP-297 is a potent FXR agonist *in vitro*

FXR Assays	EDP-297 (EC_{50}; nM)
CHO cell reporter assay GAL4-FXR chimera	0.18 ± 0.10
FXR co-activator binding assay TR-FRET	4.5 ± 1.7
FXR target gene regulation HepaRG (BSEP)	0.62 ± 0.20
FXR target gene regulation Primary human hepatocytes (SHP)	0.20 ± 0.20
Selectivity TGR5 GPCR activity assay	>15000
Nuclear receptor panel	Selective for FXR at 10μM

Table 1. *In vitro* characteristics of EDP-297.

RESULTS

EDP-297 Modulates Inflammatory and Lipid Metabolism Pathways in a 3D Human Liver Microtissue NASH Model

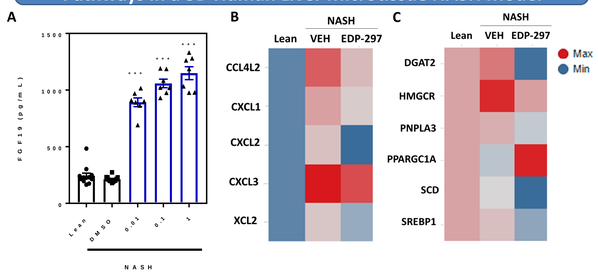

Figure 1. A. EDP-297 (0.01, 0.1, 1 μM) increases FGF19 production in 3D human microtissues. One-way ANOVA followed by Dunnett's multiple comparisons test vs NASH DMSO (n=7/group). ***p<0.001. Heat maps of RNA-seq show EDP-297 (1 μM) regulates inflammation (**B**) and lipid metabolism (**C**) in3D human microtissues. All genes shown have p<0.05 vs NASH Vehicle (Veh).

EDP-297 Protects Rats from Bile Duct Ligation (BDL) -Induced Liver Injury

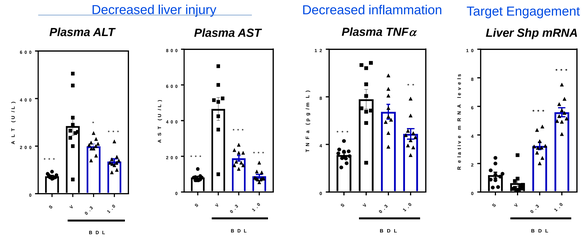

Figure 2. EDP-297 decreases plasma ALT, AST and TNFα levels in a dose-dependent manner. S = sham; V = BDL vehicle; 0.3, 1.0 = Dose of EDP-297 (mg/kg). One-way ANOVA followed by Dunnett's multiple comparisons test vs BDL vehicle (n=10/group). *<p<0.05, **p<0.01, ***p<0.001.

EDP-297 Modulates Inflammatory, Fibrotic, Apoptotic and Bile Acid Transport Pathways in BDL Rats

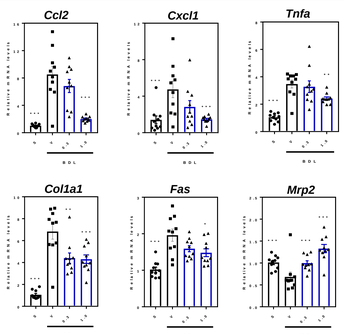

Figure 3. Effects of EDP-297 on expression of genes involved in inflammatory, fibrotic, apoptotic and bile acid transport pathways in BDL rats. S = sham; V = BDL vehicle; 0.3, 1.0 = Dose of EDP-297 (mg/kg). One-way ANOVA followed by Dunnett's multiple comparisons test vs BDL vehicle (n=10/group). *<p<0.05, **p<0.01, ***p<0.001.

RESULTS

EDP-297 Reduces Hepatocellular Degeneration/Necrosis and Collagen in BDL Rats

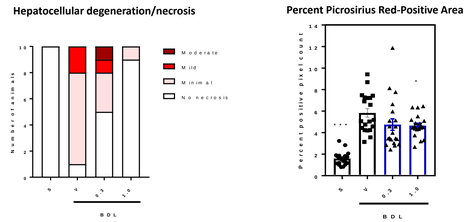

Figure 4. Histopathological scoring for hepatocellular degeneration/necrosis and collagen levels (positive pixel counts) were determined on H&E- and Picrosirius red-stained slides, respectively. EDP-297 reduced necrosis and collagen levels in BDL rats. S = sham; V = BDL vehicle; 0.3, 1.0 = Dose of EDP-297 (mg/kg). One-way ANOVA followed by Dunnett's multiple comparisons test vs BDL vehicle (n=10/group). *<p<0.05, **p<0.01

EDP-297 Exerts Hepatoprotective Effects in a Diet-Induced Obese NASH (DIO-NASH) Mouse Model

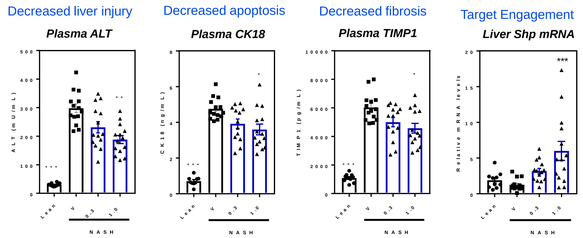

Figure 5. EDP-297 decreases plasma ALT, CK18 and TIMP1 levels in DIO-NASH mice. V = NASH vehicle; 0.3, 1.0 = Dose of EDP-297 (mg/kg). One-way ANOVA followed by Dunnett's multiple comparisons test vs NASH vehicle (n=10/lean; 14/all other groups). *<p<0.05, **p<0.01, ***p<0.001.

EDP-297 Regulates Inflammatory, Apoptotic and Fibrotic Pathways in DIO-NASH Mice

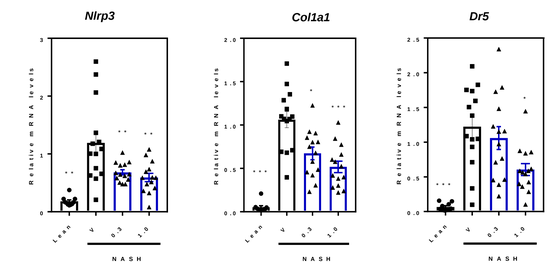

Figure 6. Effects of EDP-297 on expression of genes involved in inflammatory, fibrotic, and apoptotic pathways in DIO-NASH mice. V = NASH vehicle; 0.3, 1.0 = Dose of EDP-297 (mg/kg). One-way ANOVA followed by Dunnett's multiple comparisons test vs NASH vehicle (n=10/lean; 14/all other groups). *<p<0.05, **p<0.01, ***p<0.001.

RESULTS

EDP-297 Reduces Galectin-3 and Collagen in DIO-NASH Mice

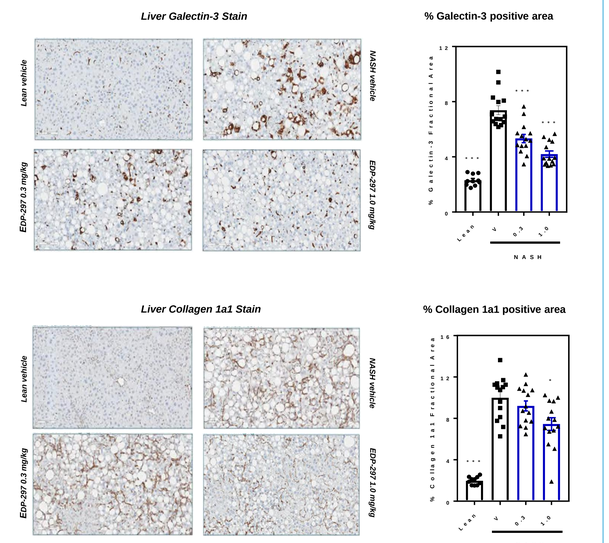

Figure 7. Histopathological analysis for galectin-3 and collagen levels were determined on galectin-3 and collagen 1a1-stained slides. EDP-297 reduced galectin-3 and collagen levels in DIO-NASH mice. V = NASH vehicle; 0.3, 1.0 = Dose of EDP-297 (mg/kg). One-way ANOVA followed by Dunnett's multiple comparisons test vs NASH vehicle. *<p<0.05, **p<0.01.

CONCLUSION

- EDP-297 is a potent and selective FXR agonist.
- EDP-297 modulates lipid metabolism and inflammatory pathways in 3D human liver microtissues.
- EDP-297 treatment ameliorates liver injury, inflammation and necrosis in BDL rats.
- EDP-297 exerts hepatoprotective effects in reducing liver injury, inflammation and fibrosis in diet-induced obese NASH mice.
- These data support further evaluation of EDP-297 for the treatment of NASH.

ACKNOWLEDGEMENTS

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