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EP-024297, A Novel and Selective Farnesoid X Receptor Agonist, Exhibits High Potency and Efficacy *In Vitro* and *In Vivo*

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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). NASH is a leading cause of cirrhosis and hepatocellular carcinomas. 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]. To date, no optimal treatments are available, further underscoring the need for effective therapies.

Bile acids act as dietary detergents and signaling molecules which, through binding nuclear and membrane receptors such as farnesoid X receptor (FXR) and transmembrane G-protein coupled receptor (TGR5), play key roles in regulating energy homeostasis. 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, directly or through the transcription factor small heterodimer partner (SHP), 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]. The design of FXR agonists was therefore undertaken to identify novel FXR agonists for NASH.

Methods

In vitro efficacy, TGR5 activation and cytotoxicity

EP-024297, an FXR agonist, and obeticholic acid (OCA) were tested side-by-side for potency (EC_{50}) and efficacy using a chimeric FXR reporter assay in Chinese hamster ovary cells. Activation of TGR5 was assessed by measuring cellular levels of cyclic adenosine monophosphate using a competitive immunoassay. Cell cytotoxicity (CC50) was determined by measuring ATP levels using the ATPlite 1step kit. The in vitro safety index was calculated by CC50 over EC50.

Plasma and tissue concentration measurements

Plasma and tissue concentrations of EP-024297 were determined by an LC-MS/MS assay.

Cell Culture

The human hepatoma cell line, Huh7, was treated with EP-024297 or OCA at various concentrations.

Animal studies and gene expression analysis

The *in vivo* activity of EP-024297 was determined in C57BL/6 mice treated by oral gavage with compounds at the indicated doses for three days. Gene expression analysis was determined by RT-PCR.

Results

Table 1. EP-024297 is a highly potent FXR agonist. EP-024297 was 20,000-fold more potent than OCA (EC_{50}: 569 nM) in the FXR reporter assay with an EC_{50} value of 0.026 nM, and had minimal activity against TGR5.

Compound	Chimeric FXR activation (CHO)			TGR5 activation (CHO)		
	N	EC_{50} (nM)	Efficacy (%)	N	EC_{50} (nM)	Efficacy(%)
OCA	198	569 ± 96	213% ± 39%	56	381 ± 102	72% ± 11%
EP-024297	4	0.026 ±0.012	276% ± 8%	2	>15,000	NA

Fig 1. EP-024297 dose-dependently increases SHP and BSEP expression in Huh7.5 cells. At 0.03 nM, close to the EC_{50} value for EP-024297 in the reporter assay, EP-024297 induced a 4-fold increase in SHP expression, whereas OCA had no effect.

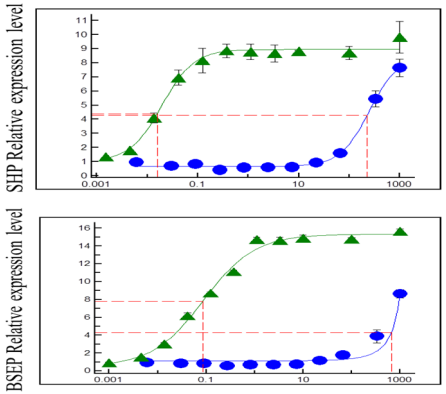
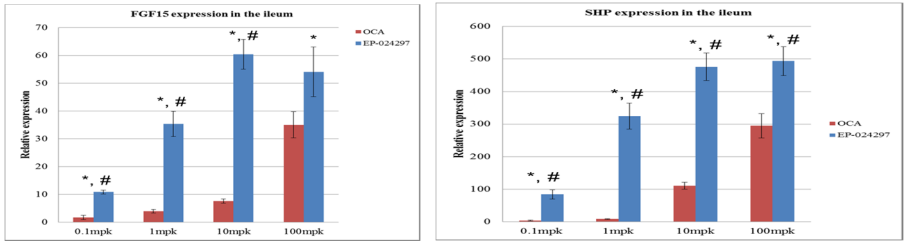


Table 2. Mouse plasma and tissue concentrations of EP-024297

Dose (mg/kg)	Plasma (ng/mL)	Liver (ng/g)	Intestine (ng/g)
0.1	BLQ	BLQ	BLQ
1	6.3 ±5.8	450.3 ±249.6	1587.5 ±1005.2
10	21.7 ±0.5	2193.1 ±1059.3	4014.9 ±1443.3
100	632.0 ±535.4	20048.9 ±5470.7	72297.3 ±30914.0

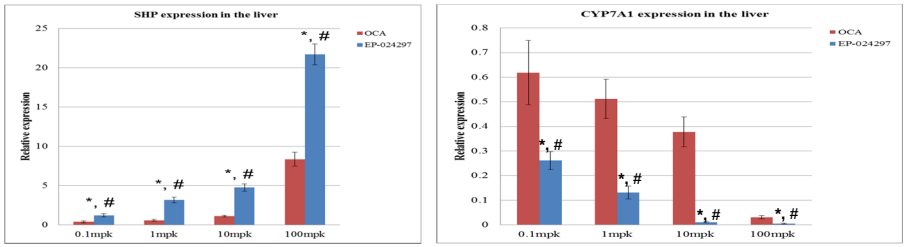
BLQ: below limit of quantitation

Fig 2. EP-024297 dose-dependently increases ileal FGF15 and SHP expression in mouse. At a dose of 0.1 mg/kg, EP-024297 significantly increased FGF15 and SHP expression by 11- and 84-fold respectively, while OCA induced 3 and 2-fold increases.



** p-value < 0.05 between EP-024297 and vehicle control; # p<0.05 between OCA and EP-024297*

Fig 3. EP-024297 exerts greater potency than OCA in induction of SHP and repression of CYP7A1 expression in the mouse liver. EP-024297 significantly increased SHP and decreased CYP7A1 expression maximally by 22-fold and 200-fold respectively.



** p-value < 0.05 between EP-024297 and vehicle control; # p<0.05 between OCA and EP-024297*

Conclusions

EP-024297 is a highly potent FXR agonist with minimal activity against TGR5. While OCA showed dual-agonist activity against both FXR and TGR5, EP-024297 was 20,000 fold more potent in FXR activation. Consistent with reporter assay results, EP-024297 demonstrated significantly greater potency and efficacy than OCA in the regulation of FXR target genes in bile acid metabolism *in vitro* and *in vivo*. These results warrant further investigation of EP-024297 as a potential therapy for NASH.

Acknowledgement

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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.