Liver-distributed Farnesoid X Receptor agonist TERN-101 is more efficacious in a mouse model of non-alcoholic steatohepatitis than Obeticholic Acid

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1 INTRODUCTION

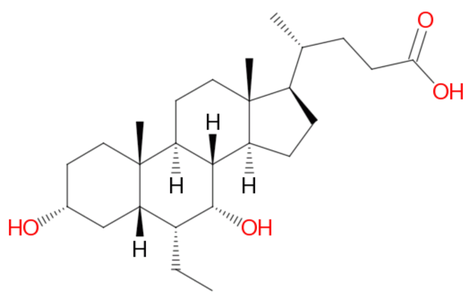
Farnesoid X Receptor (FXR) is a nuclear hormone receptor that controls the conversion of cholesterol into bile acids and maintains homeostasis of multiple metabolic pathways.

FXR is critical for liver and intestinal function but may regulate these organs differently due to a non-overlapping set of FXR binding sites in the two tissues.

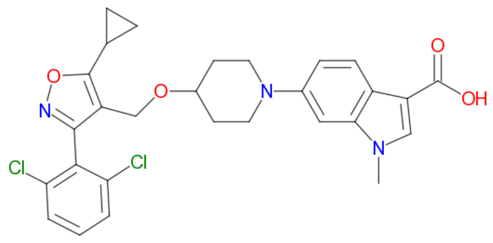
The FXR agonist obeticholic acid (OCA), a synthetically modified bile acid, reduced liver fibrosis in patients with non-alcoholic steatohepatitis (NASH) (REGENERATE trial).

TERN-101, a novel liver-distributed non-steroidal agonist of FXR, is currently in Phase 2 development for NASH.

Obeticholic Acid



TERN-101

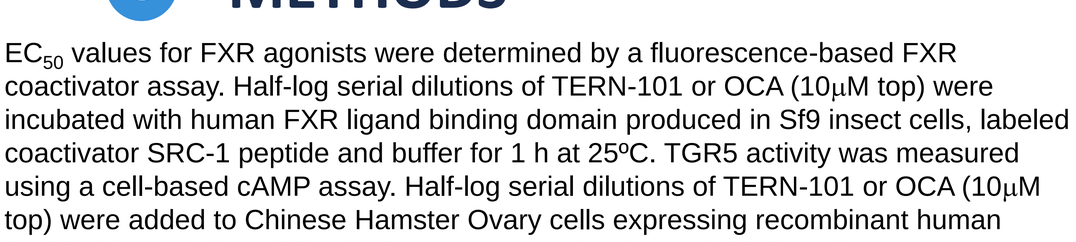


2 AIM

To compare the efficacy of TERN-101 and OCA in a mouse model of NASH

3 METHODS

- EC_{50} values for FXR agonists were determined by a fluorescence-based FXR coactivator assay. Half-log serial dilutions of TERN-101 or OCA (10μM top) were incubated with human FXR ligand binding domain produced in Sf9 insect cells, labeled coactivator SRC-1 peptide and buffer for 1 h at 25ºC. TGR5 activity was measured using a cell-based cAMP assay. Half-log serial dilutions of TERN-101 or OCA (10μM top) were added to Chinese Hamster Ovary cells expressing recombinant human TGR5. After 30min at RT, cAMP was measured using an HTRF readout.
- EC_{50} values for FXR-regulated gene expression were determined using a cell-based RNA assay. Half-log serial dilutions of TERN-101 or OCA (3μM top) were added to human Huh7 hepatoma cells. After 11 h at 37ºC, RNA was isolated and analyzed by RT-qPCR using primers to small heterodimer partner (SHP), bile salt export pump (BSEP) and fibroblast growth factor 19 (FGF19).
- TERN-101 and OCA tissue distribution in SD rats (n=3 rats/compound) was determined following a 30 min IV infusion at 2 mg/kg (mpk) for each compound. Blood and liver tissue samples were collected at 2 h post dose to determine tissue/plasma ratio. Drug was quantitated using LC-MS/MS. For tissue gene expression study, C57BL/6 mice (n=6/cohort) were given a single oral dose of vehicle, TERN-101 (10 mg/kg), or OCA (30 mg/kg). Tissue RNA was collected at termination and analyzed by RT-qPCR. For RT-qPCR, gene-specific primers were used to quantitate FXR-regulated gene expression in liver and ileum using the 2-ΔΔCT method.
- Male C57/Bl6J mice were fed a high fat diet (D12492, Research Diet, fat/protein/carbohydrate 60/20/20 Kcal%) for 10 weeks to induce obesity (>36g mouse) prior to biweekly intraperitoneal administration of carbon tetrachloride (CCl_4) and daily oral drug treatment. Following 28 days of TERN-101 (10 mpk) or OCA (30 mpk) dosing, serum lipids, liver enzymes, and liver tissue were analyzed for changes from vehicle control. Plasma 7-alpha-hydroxy-4-cholesten-3-one (7-α-C4) was measured as a biomarker of FXR activation. 7-α-C4 was quantitated using LC-MS/MS.



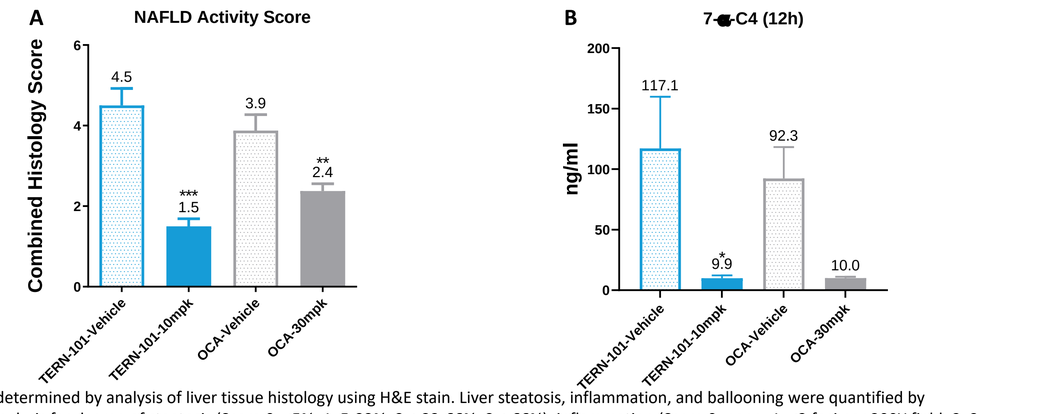
4 RESULTS

Table 1. TERN-101 is a potent and selective FXR agonist

Assay	TERN-101 EC_{50} (nM)	OCA EC_{50} (nM)
FXR Agonist	57	73
TGR5 Agonist	>10,000	770
SHP Gene Induction/Huh7	50	200
BSEP Gene Induction/Huh7	40	200
FGF19 Gene Induction/Huh7	30	130

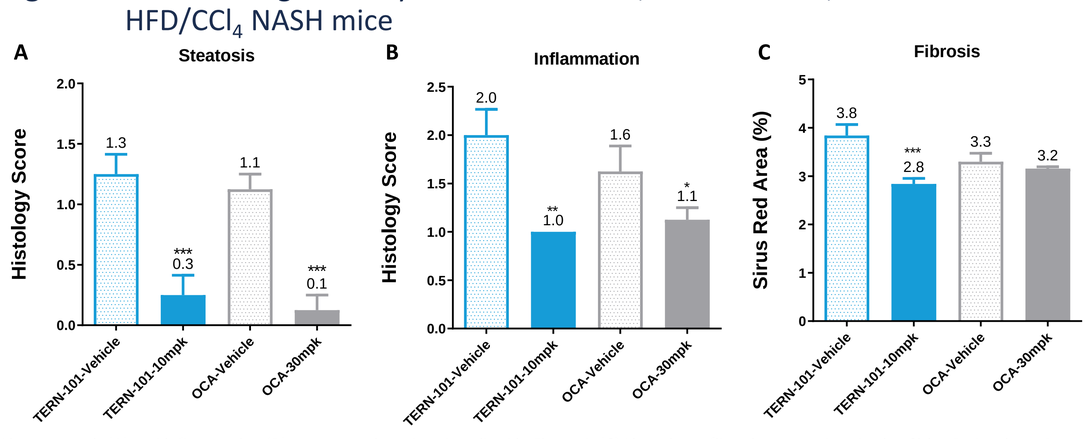
4 RESULTS

Figure 1. TERN-101 and OCA reduce NAFLD Activity Score (NAS) and 7α-Hydroxy-4-cholesten-3-one (7-α-C4) in HFD/CCl_4 NASH mice



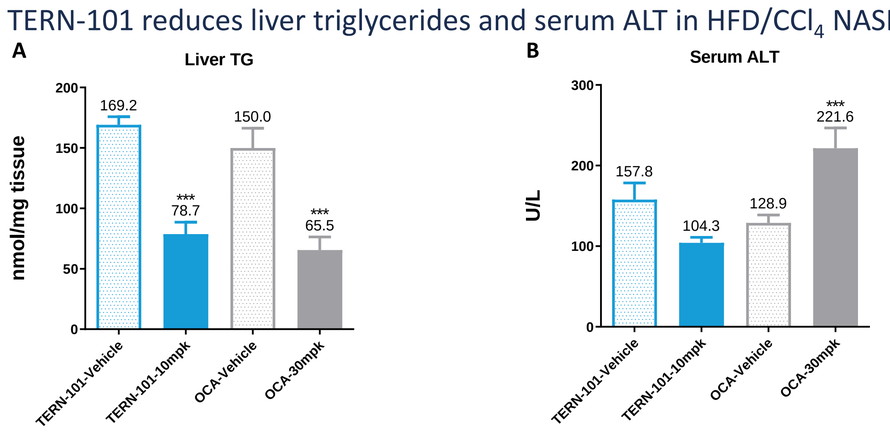
(A) NAS was determined by analysis of liver tissue histology using H&E stain. Liver steatosis, inflammation, and ballooning were quantified by histological analysis for degree of steatosis (Score 0, <5%; 1, 5-33%; 2, >33-66%; 3, >66%), inflammation (Score 0, none; 1, <2 foci per 200X field; 2, 2-4 foci per 200X field; 3, >4 foci per 200X field), and ballooning (Score 0, none; 1, few ballooning hepatocytes; 2, many hepatocytes with prominent ballooning). Data are presented as Mean ± SEM (n=8); ***p＜0.001, **p＜0.01 vs Vehicle; statistics determined by one-way ANOVA followed by Tukey. (B) Plasma was collected at 12 h post drug dosing on day 23 and analyzed for 7-α-C4 using LC-MS/MS. Data are presented as Mean ± SD (n=3); *p＜0.05 vs Vehicle; statistics determined by one-way ANOVA followed by Tukey.

Figure 2. TERN-101 significantly reduces steatosis, inflammation, and fibrosis in HFD/CCl_4 NASH mice



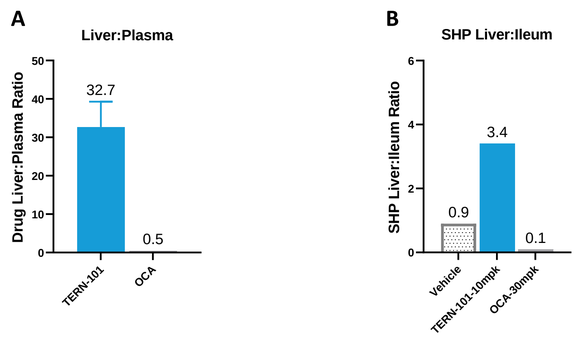
Liver steatosis (A) and inflammation (B) were quantitated as described in Figure 1. (C) Liver tissue fibrosis was analyzed using Sirius Red stain and quantified by histological analysis for the percentage of Sirius Red-positive liver sections. Data are presented as Mean ± SEM (n=8); ***p＜0.001, **p＜0.01, *p＜0.05 vs Vehicle; statistics determined by one-way ANOVA followed by Tukey.

Figure 3. TERN-101 reduces liver triglycerides and serum ALT in HFD/CCl_4 NASH mice



(A) Liver triglyceride was measured from liver tissue using an automatic biochemical analyzer. (B) Serum at termination was analyzed for alanine aminotransferase (ALT), using an automatic biochemical analyzer. Data are presented as Mean ± SEM (n=8); ***p＜0.001 vs Vehicle; statistics determined by one-way ANOVA followed by Tukey.

Figure 4. TERN-101 is a liver-distributed FXR agonist and induces higher liver gene expression relative to OCA



(A) Liver to plasma distribution of TERN-101 and OCA in rat was measured using LC-MS/MS. Data are presented as mean ratio value ± SD (n=3). (B) Small heterodimer partner (SHP) gene expression in mouse liver and ileum was analyzed using RT-qPCR. The ratio of SHP liver to ileum gene expression is shown. Data are presented as mean ratio value (n=6).

5 CONCLUSIONS

- TERN-101 is a potent liver-distributed FXR agonist.
- TERN-101 strongly suppressed liver steatosis, inflammation, and fibrosis in a diet-induced obese mouse model of NASH.
- On a per dose basis, TERN-101 appears to be more efficacious in the DIO-CCl_4 mouse NASH model than OCA.
- The higher efficacy of TERN-101 may be due to its high liver distribution and effects on hepatic FXR activation.

6 DISCLOSURES/CONTACT

Authors are employees and stockholders in Terns Pharmaceuticals

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