A novel and highly potent FXR agonist EDP-305 suppresses liver injury and fibrosis in a murine model of steatohepatitis

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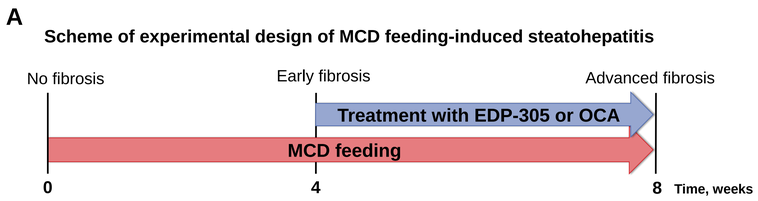
BACKGROUND & AIMS: Farnesoid X receptor (FXR) agonism is a promising strategy to treat chronic liver diseases such as non-alcoholic steatohepatitis (NASH). EDP-305 is a novel and potent FXR agonist with a single-digit nanomolar activity in vitro. It is highly selective for FXR with no/minimal cross-reactivity to the G protein-coupled bile acid receptor 1 (TGR5) or other nuclear receptors. Herein we report therapeutic efficacy of EDP-305 in mice with steatohepatitis and fibrosis, in direct comparison with the first-in-class FXR agonist, obeticholic acid (OCA).

METHODS: Steatohepatitis was induced in C57Bl/6 mice with a methionine-choline deficient diet (MCD). Delayed treatments were administered between 4 weeks (steatohepatitis with incipient fibrosis) and 8 weeks (advanced steatohepatitis with advanced fibrosis) on MCD (n=7-12/group, Figure 1). Two doses of EDP-305 (10 and 30 mg/kg) or vehicle were administered via daily oral gavage. A parallel group received OCA (30mg/kg/day p.o.) as a comparator. Liver injury and progression of liver fibrosis were evaluated by serum chemistry, histology, and biochemical determination of collagen.

RESULTS: No apparent adverse effects of treatments were noted during the study. Serum levels of transaminases ALT and AST were both significantly decreased (by 62% and 37%, respectively) in MCD-fed mice receiving 30mg/kg EDP-305 compared to vehicle controls. Mice receiving low dose 10 mg/kg EDP-305 and obeticholic acid (30mg/kg) showed a clear trend towards lower ALT/AST levels compared to vehicle control, but these changes did not reach statistical significance. Total bilirubin levels were not significantly affected by any of the treatments (Figure 2A). EDP-305 at both doses (10 and 30 mg/kg) had a profound inhibitory effect on liver fibrosis progression, with up to 70% reduction in hepatic collagen deposition ($p<0.05$, ANOVA) as determined biochemically via hydroxyproline measurement (Figure 2B). Histologically, MCD-fed control mice developed the advanced perisinusoidal fibrosis ("chicken wire") characteristic of NASH. Treatment with EDP-305 was associated with markedly reduced periosinusoidal fibrosis compared to placebo group. OCA (30 mg/kg) did not have an appreciable effect on hepatic hydroxyproline levels and connective tissue histology (Figure 3).



B

Groups	n=	Final BW, g	Liver/BW *100, %	Spleen/BW *1000)
CTRL (healthy)	8	22.640.71	4.84±0.25	3.07±0.14
MCD 4w Start	10	15.97±0.64	4.38±0.13	2.46±0.10
Placebo	12	13.57±0.39	4.41±0.14	2.28±0.09
EDP-305 10mpk	7	14.60±0.37	4.71±0.13	2.14±0.09
EDP-305 30mpk	10	14.70±0.23	5.13±0.22*	2.61±0.22
OCA 30mpk	9	14.86±0.29	5.18±0.17*	2.50±0.11

Figure 1. Scheme of experiment and group design of MCD feeding-induced steatohepatitis model. (A) Steatohepatitis was induced in male 8 weeks old C57Bl6 mice for 8 weeks. Relevant time- points have been established that reflect disease progression: 4 weeks (advanced steatohepatitis with early fibrosis) and 8 weeks (steatohepatitis with advanced fibrosis) on MCD diet. Treatment started after 4 weeks of MCD feeding, when steatohepatitis and incipient fibrosis (histologically) were already established, and continued for the following 4 weeks. (B) Animal numbers and groups, body weight (BW), liver and spleen weights (relative to body weight, mean±std. error). Oral administration of EDP-305 or OCA did not have any effect on body weight. Liver weight relative to the body size was higher in mice receiving high dose of both EDP-305 or OCA ($p<0.05$, ANOVA with Dunett's post-test) compared to placebo.

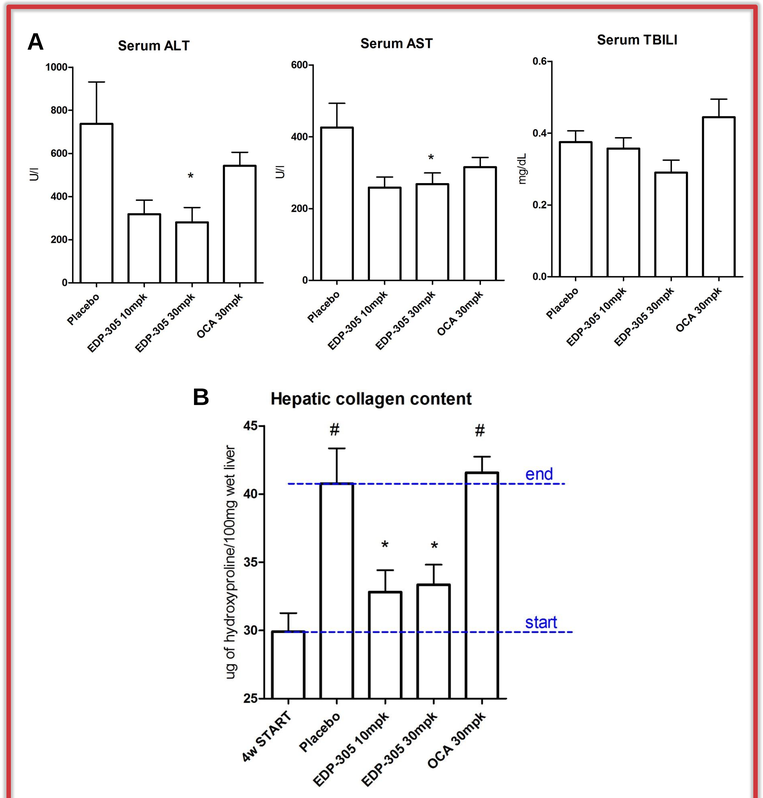


Figure 2. Effect of treatments on liver function tests and hepatic collagen deposition in MCD-fed mice. (A) Serum levels of transaminases (ALT and AST) were significantly decreased in mice receiving 30mg/kg EDP-305 compared to vehicle controls. Mice receiving low dose 10 mg/kg EDP-305 and obeticholic acid (OCA 30mg/kg) showed only a trend towards lower ALT/AST levels compared to vehicle control (n.s.). (B) EDP-305 at both doses significantly suppressed collagen deposition (determined biochemically via hydroxyproline content) compared to placebo group, whereas OCA at 30mg/kg did not. ANOVA with Dunnett's post-test: *, $p<0.05$ compared to placebo control group, #, $p<0.05$ compared to start of treatment control group.

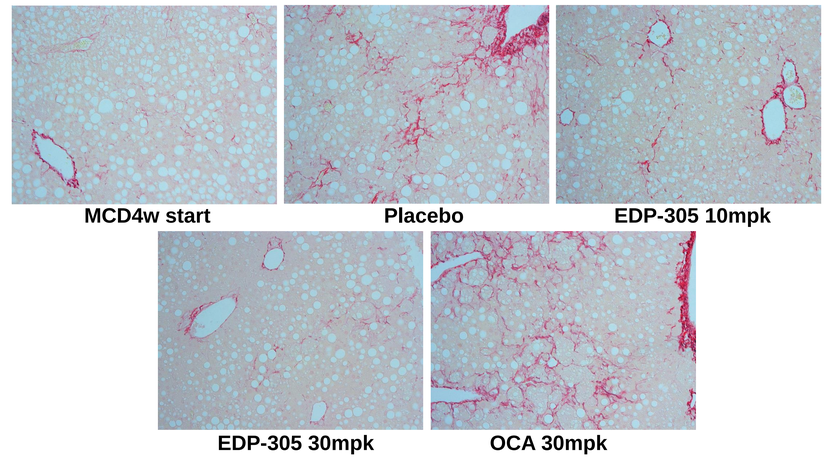


Figure 3. Effect of treatments on fibrosis (assessed histologically via picrosirius red staining) in MCD-fed mice (A) Connective tissue staining fibrillar collagen stained red) showed significant progression of metabolic-type sinusoidal fibrosis ("chicken wire") characteristic of NASH from minimal (week 4 on MCD, "MCD4w start" group) to significant (week 8 on MCD, "Placebo" treatment group) in placebo-treated MCD- fed mice. Sinusoidal fibrosis appeared markedly suppressed in MCD-fed mice receiving EDP-305 at 10 and 30 mg/kg, but not in OCA-treated group. Representative images of connective tissue staining of livers are shown (original magnification 200x)

CONCLUSIONS:

Treatment with the novel FXR agonist EDP-305 potently improved pre-established liver injury and hepatic fibrosis (assessed biochemically and histologically) in an MCD-induced model of steatohepatitis in mice. By all studied parameters of liver injury and fibrosis, EDP-305 outperformed the first in class FXR agonist, obeticholic acid.