A novel FXR agonist EDP-305 potently suppresses liver injury and fibrosis in mouse models of biliary and metabolic liver disease

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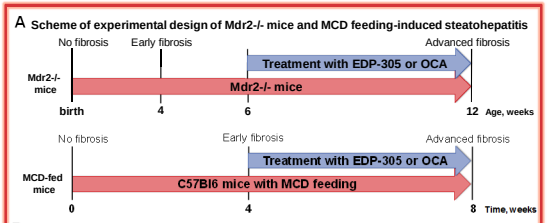
BACKGROUND & AIMS: EDP-305 is a novel and potent FXR agonist with a single-digit nanomolar affinity in vitro, with no/minimal cross-reactivity to the G protein-coupled bile acid receptor 1 (TGR5) or other nuclear receptors. Herein we report therapeutic efficacy of EDP-305, in direct comparison with the first-in-class FXR agonist obeticholic acid (OCA) in two mouse models: 1) Mdr2-/- model with progressive biliary-type (periportal) fibrosis resembling that observed in PSC, PBC, cystic fibrosis-related liver disease CFLD and congenital biliary cirrhosis; and 2) MCD model of steatohepatitis with metabolic-type perisinusoidal fibrosis.

METHODS: Delayed therapy with EDP-305 (10 and 30mg/kg/day) was tested in mouse models of pre-stablished i) biliary fibrosis (BALBc.Mdr2-/-, 6 through 12 weeks of age, n=9-11/group) and ii) steatohepatitis induced by methionine/choline-deficient diet (MCD, week 4 through 8, n=8-12/group). Parallel groups received either no treatment as control or OCA (30mg/kg/day p.o.) as a comparator (Figure 1).

RESULTS: In BALBc.Mdr2-/- model of biliary fibrosis, 10 and 30 mg/kg of EDP-305 significantly reduced serum transaminase (ALT) levels by 30% and 53%, respectively compared to controls (Figure 2A).

Histologically, untreated group developed severe periportal and perisinusoidal fibrosis with bridging, which was markedly attenuated in BALBc.Mdr2-/- mice receiving EDP-305 at both doses (Fig. 3A), with up to 39% reduction in hepatic collagen content in high-dose EDP-305 (Fig. 2B, $p<0.05$, ANOVA). OCA at 30mg/kg did not improve fibrosis histologically, and had no significant impact on hepatic collagen levels or serum ALT in the BALBc.Mdr2-/- model compared to control group.

In MCD-fed mice with steatohepatitis, serum ALT were significantly decreased by 62% in the 30mg/kg EDP-305-treated group compared to controls (Fig. 2A). 10 mg/kg EDP-305 and obeticholic acid (30mg/kg) showed only a trend towards lower ALT levels (not significant). EDP-305 at both doses profoundly inhibited MCD-induced liver fibrosis, with up to 70% reduction in hepatic collagen deposition (Fig. 2B, $p<0.05$, ANOVA). Histologically, MCD-fed control mice developed the advanced perisinusoidal "chicken wire" fibrosis, which was markedly reduced by EDP-305 compared to the control group (Fig. 3A). OCA (30 mg/kg) did not have an appreciable effect on hepatic hydroxyproline levels and connective tissue histology in the MCD model.



B

	Groups	n=	Final BW, g	Liver/BW *100, %	Spleen/BW *1000)
Mdr2-/- mice	WT BALBc (healthy)	5	21.40±0.37	5.40±0.09	3.87±0.11
	KO 6w Start	5	15.86±1.24	7.93±0.09	7.74±0.20
	Placebo	11	23.43±0.37	7.24±0.15	8.78±0.16
	EDP-305 10mpk	9	22.71±0.46	6.63±0.25	8.06±0.21
	EDP-305 30mpk	10	22.89±0.44	7.43±0.20	8.16±0.24
	OCA 30mpk	5	22.90±0.48	7.88±0.37	11.66±0.49*
C57Bl6 mice with MCD feeding	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 Mdr2-/- biliary-type fibrosis model and MCD feeding-induced steatohepatitis model. (A) Mdr2-/- mice were chosen as biliary fibrosis model which progressive liver fibrosis occurred since 4 weeks mice. Treatment started after 6 weeks mice and continued for the following 6 weeks. 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.

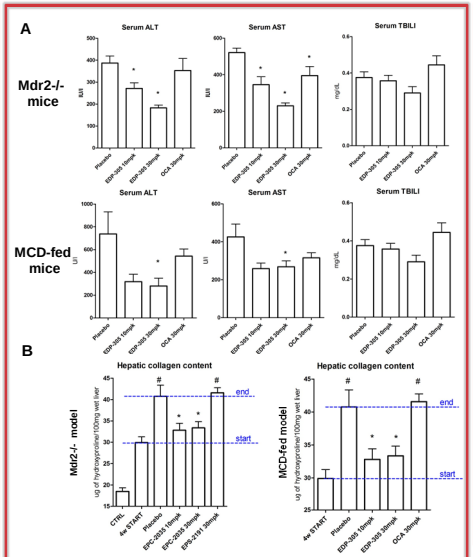


Figure 2. Effect of treatments on liver function tests and hepatic collagen deposition in Mdr2-/- mice and MCD-fed mice. (A) Serum levels of transaminases (ALT and AST) were significantly decreased in Mdr2-/- mice and MCD-fed mice receiving 30mg/kg EDP-305 compared to vehicle controls. Mice receiving low dose 10 mg/kg EDP-305 and obeticholic acid (OCA 30mg/kg) showed only a trend towards lower TBIL levels compared to vehicle control (n.s.). (B) In Mdr2-/- model and MCD-fed model, 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.

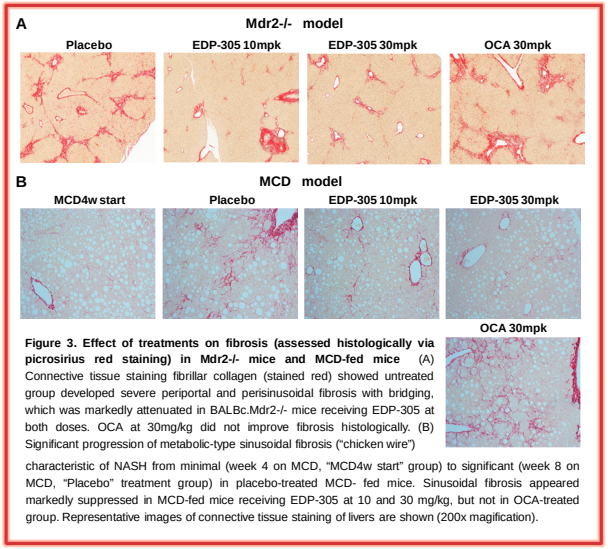


Figure 3. Effect of treatments on fibrosis (assessed histologically via picrosirius red staining) in Mdr2-/- mice and MCD-fed mice (A) Connective tissue staining fibrillar collagen (stained red) showed untreated group developed severe periportal and perisinusoidal fibrosis with bridging, which was markedly attenuated in BALBc.Mdr2-/- mice receiving EDP-305 at both doses. OCA at 30mg/kg did not improve fibrosis histologically. (B) 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 (200x magification).

CONCLUSIONS:

Treatment with the novel FXR agonist EDP-305 potently improved pre-established liver injury and hepatic fibrosis in both biliary (BALBc.Mdr2-/-) and metabolic (MCD) models of liver disease in mice. In both models and by all studied parameters of liver injury and fibrosis, EDP-305 outperformed the first-in-class FXR agonist, obeticholic acid.