A novel FXR agonist EDP-305 potently suppresses hepatic stellate cell activation and hepatic fibrosis in chronic 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 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 hepatic stellate cell (HSC) cultures and in two mouse models of pre-established biliary and non-biliary liver fibrosis.

METHODS: Effects of EDP-305 were studied in vitro in primary murine hepatic stellate cells (HSC) cultures. Delayed therapy with EDP-305 (10 and 30mg/kg/day) was tested in i) BALBc.Mdr2-/- model with progressive biliary-type (periportal) fibrosis resembling that observed in PSC, PBC and congenital biliary cirrhosis, and ii) methionine/choline-deficient diet (MCD)-induced steatohepatitis model with metabolic-type peri-sinusoidal fibrosis. Parallel groups received either no treatment as control or OCA (30mg/kg/day) as a comparator.

RESULTS: In vitro, 24h incubation with 50-500nM EDP-305 dose-dependently suppressed cell proliferation and fibrogenic mRNA expression (COL1A1, TGFb1 and TIMP-1) in freshly isolated HSC (Figure 1).

In BALBc.Mdr2-/- model (Figure 2), serum transaminase (ALT) levels were reduced by 30% and 53% in groups treated with 10 and 30 mg/kg of EDP-305, respectively, compared to controls. Histologically, untreated group developed severe peri-portal and peri-sinusoidal fibrosis with bridging, which was markedly attenuated in BALBc.Mdr2-/- mice receiving EDP-305 at both doses, with up to 39% reduction in hepatic collagen content in high-dose EDP-305 ($p<0.05$, ANOVA). OCA at 30mg/kg did not improve fibrosis biochemically or histologically compared to control group. In MCD-fed mice with steatohepatitis (Figure 3), serum ALT was reduced by 62% in the 30mg/kg EDP-305-treated group, but not 10 mg/kg EDP-305 or OCA (30mg/kg) groups compared to controls. EDP-305 at both doses profoundly inhibited MCD-induced liver fibrosis, with up to 70% reduction in hepatic collagen ($p<0.05$, ANOVA). Histologically, MCD-fed control mice developed the advanced peri-sinusoidal "chicken wire" fibrosis, which was markedly reduced by EDP-305 compared to the control group. OCA (30 mg/kg) did not have an appreciable effect on hepatic hydroxyproline levels and connective tissue histology.

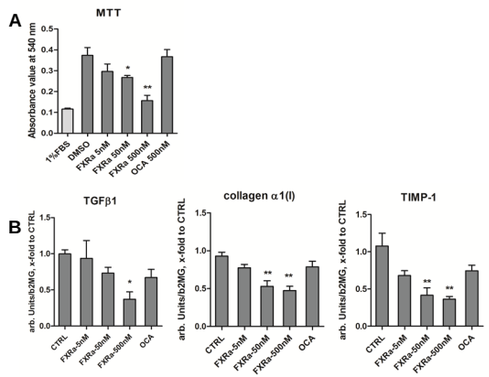

Figure 1. EDP-305 dose-dependently inhibits fibrogenic activation of primary HSC in vitro. (A) EDP-305 inhibits primary HSC proliferation (MTT assay). (B) EDP-305 significantly inhibits fibrogenic mRNA expression (TGFb1, COL1A1 and TIMP-1) in freshly isolated HSC. *, $p<0.05$; **, $p<0.01$ (ANOVA)

CONCLUSIONS:

Treatment with the novel FXR agonist EDP-305 directly inhibits HSC activation and potently improved pre-established liver injury and hepatic fibrosis in 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 compared favorably to the first-in-class FXR agonist, OCA.

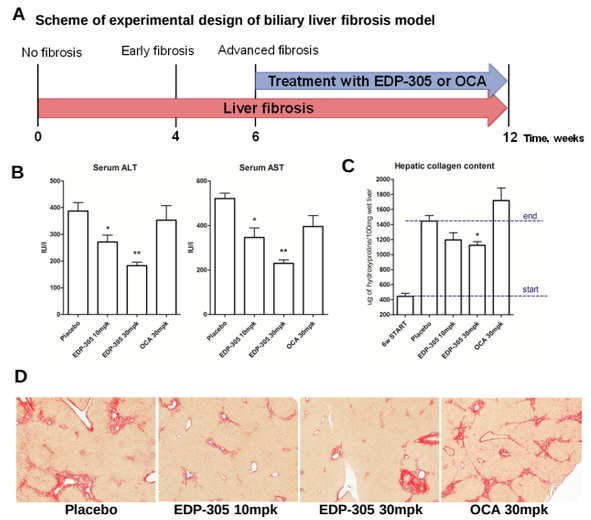

Figure 2. Therapeutic efficacy of delayed treatment with EDP-305 in BALBc.Mdr2-/- mouse model of biliary cirrhosis. (A) Scheme of experiment and group design. (B) Serum levels of transaminases (ALT and AST) were significantly decreased in mice receiving 30mg/kg EDP-305 compared to placebo controls. Mice 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 placebo control (n.s.). (C) EDP-305 at high dose significantly suppressed collagen deposition (determined biochemically via hydroxyproline content) compared to placebo group, whereas OCA at 30mg/kg did not. 6w START group is a "start of treatment" control. *, $p<0.05$; **, $p<0.01$ compared to placebo control group (ANOVA with Dunnett's post-test). (D) Connective tissue staining fibrillar collagen stained red) showed bridging peri-portal and peri-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, 50x)

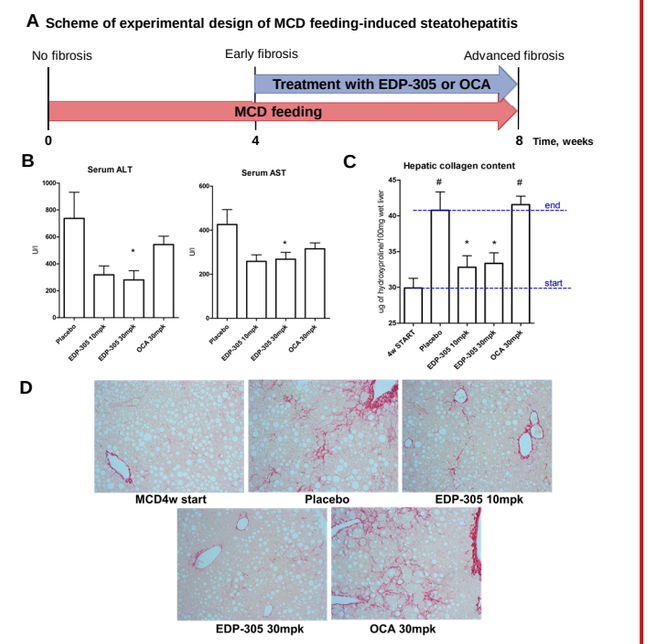

Figure 3. Therapeutic efficacy of delayed treatment with EDP-305 in mice with MCD-induced statohepatitis. (A) Scheme of experiment and group design. (B) Serum levels of transaminases (ALT and AST) were significantly decreased in mice receiving 30mg/kg EDP-305 compared to vehicle controls. (C) EDP-305 at both doses significantly suppressed collagen deposition (determined biochemically via hydroxyproline content) compared to placebo group, whereas OCA at 30mg/kg did not. 4w START group is a "start of treatment" control. *, $p<0.05$ compared to placebo control group (ANOVA with Dunnett's post-test). (D) Connective tissue staining fibrillar collagen stained red) showed sinusoidal fibrosis markedly suppressed in MCD-fed mice receiving EDP-305 at 10 and 30 mg/kg, but not in OCA-treated group. Representative images shown (original magnification, 200x)