The Farnesoid X Receptor (FXR) Agonist EDP-305 Reduces Interstitial Renal Fibrosis in a Mouse Model of Unilateral Ureteral Obstruction

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Abstract

Background: Farnesoid X receptor (FXR) is a nuclear receptor that has emerged as a key regulator in the maintenance of bile acid homeostasis. FXR agonists are currently under clinical investigation for the management of various clinical diseases such as primary biliary cholangitis and nonalcoholic steatohepatitis where they have been shown to reduce hepatic steatosis, inflammation, and fibrosis. However, the role of FXR in renal fibrosis remains to be established. Here, we investigate the effects of the FXR agonist EDP-305 in a mouse model of tubulointerstitial fibrosis via unilateral ureteral obstruction (UUO). **Methods:** Male C57Bl/6 mice received a UUO on their left kidney. On postoperative day 4, mice received daily treatment by oral gavage with either vehicle control (0.5% methylcellulose) or 10 or 30 mg/kg EDP-305. All animals were sacrificed on postoperative day 12. **Results:** EDP-305 dose-dependently decreased macrophage infiltration as measured by the F4/80 staining area with significant differences seen at the higher dose (4.5±0.46 vs. 1.4±0.49, p<0.01) which were associated with reduced pro-inflammatory cytokine gene expression (*Il-6* 208.3±59.46 vs. 32.53±3.28, p<0.01; *Tnf-α* 34.2±10.35 vs. 13.41±2.81, p<0.05). EDP-305 also dose-dependently reduced interstitial fibrosis as assessed by morphometric quantification of the collagen proportional area (CPA) and kidney hydroxyproline (HYP) levels with statistically significant differences observed at the higher dose (CPA 6.73±0.94 vs 2.58±0.39, p<0.01; and HYP 1504±140 vs. 1089±54, p<0.05). Finally, Yap activation, a major driver of fibrosis, increased after UUO injury and was diminished by EDP-305 treatment. Consistently, EDP-305 decreased TGF-β1-induced YAP nuclear localization in HK2 cells by increasing inhibitory YAP phosphorylation. **Conclusions:** Our results suggest that Yap inhibition may be a novel anti-fibrotic mechanism of FXR agonism and that FXR agonists could be used to treat renal fibrosis in patients with chronic kidney disease.

Characterizing the UUO model

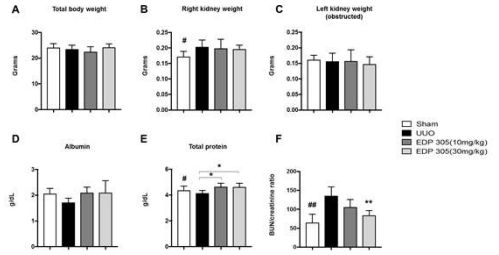

Figure 1. EDP-305 is renal protective against unilateral ureteral obstruction (UUO). At the time of sacrifice, animals were measured for **A)** total body weight, **B)** right kidney (unobstructed side) and **C)** left kidney weight (obstructed side). Renal function was evaluated by **D)** serum albumin, **E)** serum total protein, and **F)** BUN/creatinine ratio. # denotes p<0.05 and ## denotes p<0.01 compared to sham. * for p<0.05 and **<0.01 compared to UUO.

EDP-305 increases renal *Fxr* expression

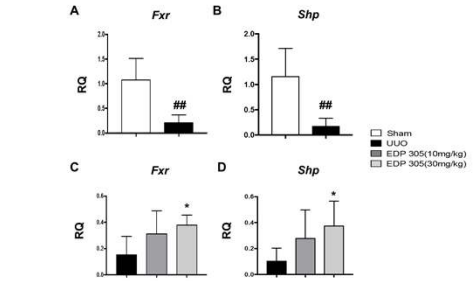

Figure 2. Unilateral ureteral obstruction (UUO) results in reduction of *Fxr* and *Shp* expression in the ligated kidney. C57BL/6 mice underwent an irreversible unilateral ureteral obstruction (UUO) procedure on the left kidney for 12 days. **A)** Farnesoid x receptor (*Fxr*) and **B)** small heterodimer partner (*Shp*) expression are significantly reduced after UUO. **C)** *Fxr* and **D)** *Shp* dose dependently increased with EDP-305 10 and 30mg/kg. ## p < 0.01 compared to Sham. * p<0.05 compared to UUO.

EDP-305 reduces inflammation

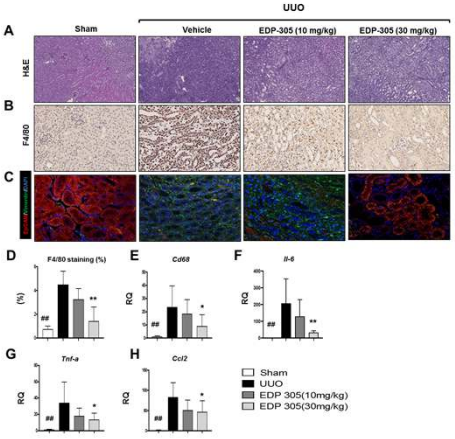

Figure 3. EDP-305 reduces inflammatory and mesenchymal activation. Representative kidney sections of sham and UUO mice treated with vehicle control (0.5% methylcellulose), EDP-305 10 or 30 mg/kg that were stained for **A)** H&E (10X magnification), **B)** F4/80 (10X magnification) or **C)** EpCAM and Vimentin (20X magnification). **D)** F4/80 staining was quantified. Expression of **E)** *Cd68*, **F)** *Il-6*, **G)** *Tnf-α*, and **H)** *Ccl-2* was assessed by real-time PCR using Taqman primers (n=8 per group). ## p < 0.01 compared to Sham. * p < 0.05 and ** p < 0.01 compared to UUO.

EDP-305 decreases interstitial fibrosis

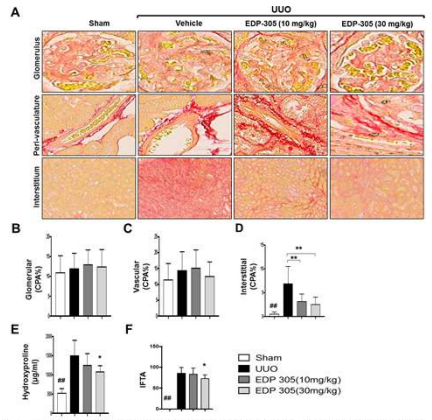

Figure 4. EDP-305 attenuates interstitial renal fibrosis. A) Representative Sirius red staining of the glomerulus (top panel; 40X magnification), vasculature (middle panel; 40X magnification), and the interstitium (lower panel; 20X magnification). Collagen proportional area (CPA) was calculated in the **B)** glomerulus, **C)** peri-vascular, and **D)** within the interstitium. **E)** Hydroxyproline content was quantified in the whole kidney. **F)** A modified interstitial fibrosis/tubular atrophy (IF/TA) score was determined by a renal pathologist as a percent of the affected area. ## denotes p<0.01 compared to Sham. * p < 0.05 and ** p < 0.01 compared to UUO.

EDP-305 decreases fibrotic markers

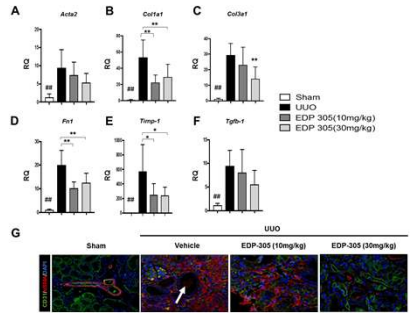

Figure 5. EDP-305 reduces renal fibrosis and protects against peri-tubular capillary loss. Expression of **A)** Actin, Alpha 2, Smooth Muscle, Aorta (*Acta2*), **B)** collagen type 1 alpha 1 (*Col1a1*), **C)** collagen type III alpha 1 (*Col3a1*), **D)** fibronectin (*Fn1*), **E)** tissue metallopetptidase inhibitor 1 (*Timp1*), **F)** transforming growth factor beta (*Tgf- β1*) was assessed with real-time PCR with Taqman primers (n=8). **G)** Representative kidney sections of UUO mice treated with EDP305 10mg/kg or 30mg/kg or vehicle control were stained for CD31 or a-SMA (20X Magnification) are shown. White arrow denotes proteinaceous debris within tubule. ## denotes p<0.01 compared to sham. * denotes p<0.05 and ** denotes p<0.01 compared to UUO.

EDP-305 reduces YAP activation

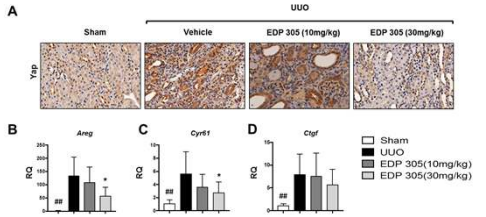

Figure 6. EDP-305 reduces nuclear activation of YAP. A) Representative kidney sections from sham or UUO mice treated with EDP 305 10mg/kg or 30mg/kg or vehicle control were stained for YAP (40X original magnification). Expression of **B)** amphiregulin (*Areg*), **C)** cysteine-rich angiogenic inducer 61 (*Cyr61*), and **D)** connective tissue growth factor (*Ctgf*) was assessed with real-time PCR with Taqman probes (n=8). ## denotes p<0.01 compared to sham. * denotes p<0.05 compared to UUO.

EDP-305 reduces YAP phosphorylation

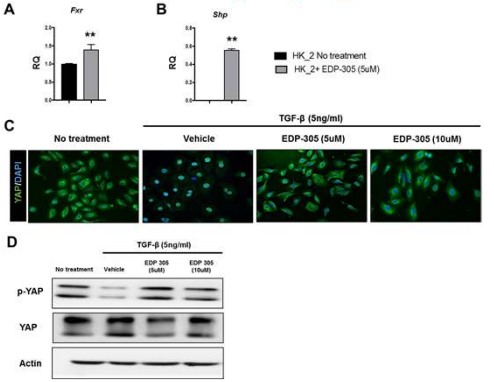

Figure 7. EDP-305 reduces nuclear activation of YAP in TGF-β stimulated human proximal tubule epithelial cells (HK-2 cells). Expression of **A)** *Fxr* and **B)** *Shp* was measured after EDP-305 stimulation. ** denotes p<0.01 compared to untreated HK-2 cells. HK-2 cells were serum starved for 24 hours and treated with 5ng/ml of TGF-β for 30 minutes in the presence or absence of FXR agonist. **C)** Immunofluorescence staining for yes-associated protein 1 (YAP). **D)** Western blot analysis was performed for the same study for phosphorylated YAP.

Conclusions

- EDP-305 is a small molecule, non-bile acid, selective FXR agonist
- EDP-305 reduced liver fibrosis in the mouse UUO model
- FXR agonists should be further evaluated for their ability to inhibit interstitial renal fibrosis