EDP-305, A Highly Selective and Potent Farnesoid X Receptor Agonist, Favorably Regulates the Expression of Key Fibrogenic Genes *In Vitro* and *In Vivo*

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INTRODUCTION

Fibrosis drives disease progression in people with advanced nonalcoholic steatohepatitis (NASH). EDP-305, a potent Farnesoid X Receptor (FXR) agonist, is currently being developed for the treatment of NASH.

AIM

Herein, we studied the anti-fibrotic activity of EDP-305 in vitro and in vivo. The effect of EDP-305 on post-transcriptional regulatory mechanism via microRNA29a (miR29a) was also characterized.

METHOD

To investigate the effects of EDP-305 on liver fibrosis in vitro, hepatic stellate cells (HSC) were induced with 5ng/ml of transforming growth factor beta (TGFβ) and co-treated with DMSO or EDP-305 for 18 hours. The in vivo anti-fibrotic effect of EDP-305 was investigated using mice with methionine/choline-deficient diet (MCD)-induced steatohepatitis and peri-sinusoidal fibrosis. 10 mg/kg of EDP-305 was orally administered to mice 4 weeks after MCD-induced early fibrosis was established and treatment duration was 4 weeks. The expression of essential genes involved in modulating the pathogenic fibrosis response associated with NASH was analyzed by RT-PCR.

RESULTS

EDP-305 significantly ($p<0.05$) decreased expression of α-smooth muscle actin (α-SMA), collagen type 1 α2 (*COL1A2*), collagen type 3 α1 (*COL3A1*), metallopeptidase inhibitor 1 (*TIMP1*) and metallopeptidase inhibitor 2 (*TIMP2*) by 68%, 42%, 57%, 80%, 65%, respectively, in the *in vitro* HSC cell culture. Consistent with the *in vitro* observation, these key fibrogenic genes were significantly down-regulated by EDP-305 in mice. Moreover, EDP-305 favorably upregulated miR29a, a crucial player in fibrosis. EDP-305 increased miR29a expression by 89% when compared to the vehicle control, which was associated with 70% reduction of hepatic collagen contents, in mice with MCD-induced fibrosis.

RESULTS (CONTINUED)

EDP-305 is a potent and selective FXR agonist

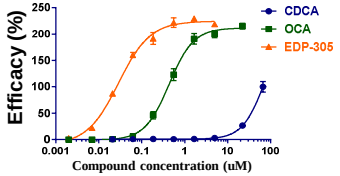


Figure 1. Activation of FXR by EDP-305. Chimeric FXR and luciferase reporter constructs were transfected into CHO cells. Luciferase signal was measured after 22 hours of treatment with CDCA (chenodeoxycholic acid), OCA (Obeticholic acid), or EDP-305.

Table 1. EDP-305 is >16-fold more potent than OCA and its major metabolites

Compound	FXR (HEK)	TGR5 (CHO)
	EC50 nM (% efficacy)*	
Obeticholic Acid (OCA)	130 (150)	380 (72)
Glyco-OCA	360 (155)	720 (157)
Tauro-OCA	250 (100)	540 (161)
EDP-305	8 (152)	> 15,000 (NS)

Table 2. EDP-305 Nuclear Receptor Selectivity

EDP-305 down-regulated inflammatory response genes

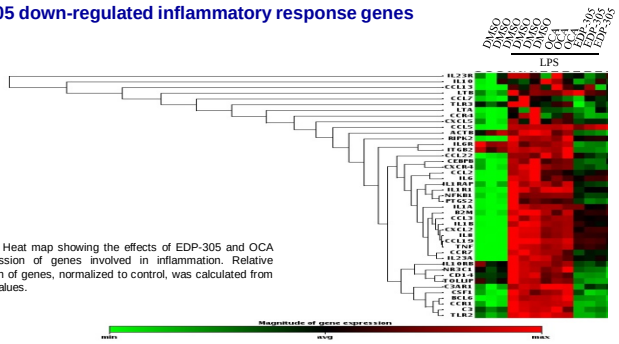


Figure 2. Heat map showing the effects of EDP-305 and OCA on expression of genes involved in inflammation. Relative expression of genes, normalized to control, was calculated from delta C_T values.

EDP-305 down-regulated multiple fibrosis-related transcripts in primary human stellate cells

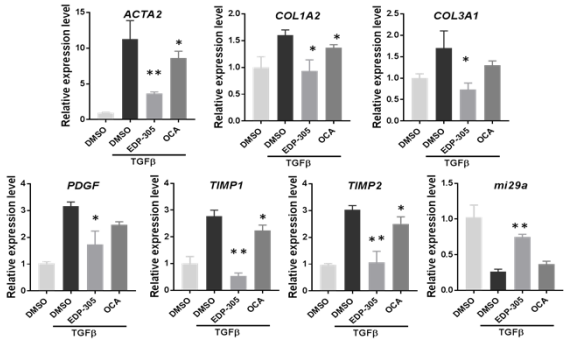
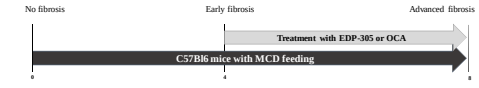


Figure 3. Activation of FXR signaling with EDP-305 significantly inhibited expression of key fibrosis genes in vitro. HSCs cells were treated with TGF-β (10 ng/ml) alone or in combination with OCA (0.5 μM) or EDP-305 (0.5 μM) for 18 hours (n=3 for each treatment). **p<0.05, and **p<0.01 vs. vehicle with two-way ANOVA.*

Scheme of experimental design of MCD feeding-induced steatohepatitis



Effect of treatments on fibrosis (assessed histologically via picrosirius red staining) in MCD-fed mice

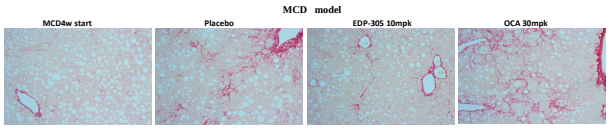


Figure 4. Effect of treatments on fibrosis (assessed histologically via picrosirius red staining) in MCD-fed. Significant progression of metabolic-type sinusoidal fibrosis ("chicken wire") characteristic of NASH from minimal (week 4 on MCD, "MCD4w start" group) to significant (week 8 on MCD, "Placebo"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

EDP-305 down-regulated multiple fibrosis-related transcripts in MCD-fed mice

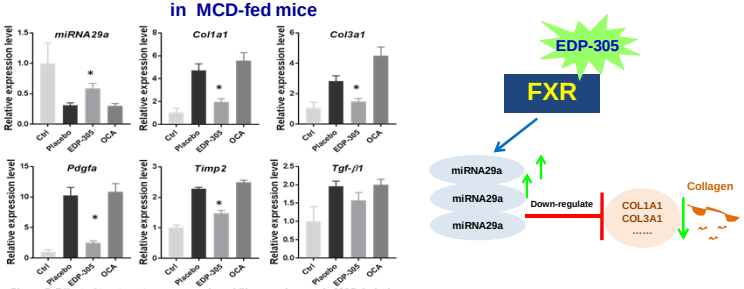


Figure 5. Effect of treatments on expression of fibrogenis gene in MCD-fed mice.
*, $p<0.05$ compared to placebo control group (ANOVA with Dunnett's post-test).

CONCLUSIONS

EDP-305 exhibits potent anti-fibrotic activity *in vitro* and *in vivo*. Moreover, EDP-305 can favorably up-regulate endogenous small non-coding RNA with a critical role in tissue fibrosis. These results warrant further clinical study of EDP-305 for the treatment of NASH.

ACKNOWLEDGEMENTS

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REFERENCES

Roles of microRNA-29a in the Antifibrotic Effect of Farnesoid X Receptor in Hepatic Stellate Cells. Mol Pharmacol. 2011 Jul; 80(1): 191-200

CONTACT INFORMATION

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