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EDP-305, A Novel and Potent Farnesoid X Receptor Agonist, Exhibits Excellent Anti-inflammatory and Anti-fibrotic Activity *In Vitro*

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Background

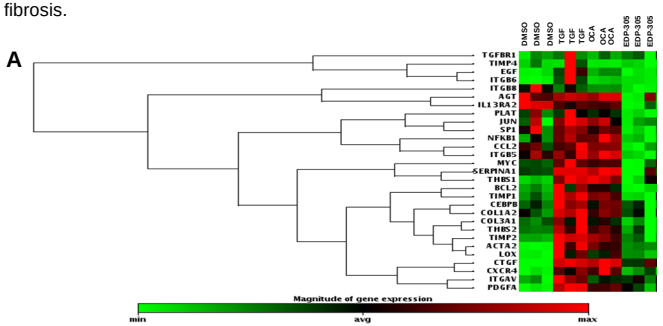
Nonalcoholic steatohepatitis (NASH) is becoming a major global health burden, with increasing prevalence and incidence worldwide. Inflammation and fibrosis play critical roles in the pathogenesis and progression of NASH [1, 2]. Herein, the anti-inflammatory and anti-fibrotic activities of EDP-305, a novel and potent Farnesoid X Receptor (FXR) agonist, were tested side-by-side with obeticholic acid (OCA).

Methods

To assess the effects of EDP-305 on genes involved in the inflammatory response, THP1 cells were treated with 50 ng/ml Lipopolysaccharides (LPS) alone, or in combination with either 50nM of EDP-305 or OCA. Hepatic stellate cells (HSC) were treated with 10 ng/ml transforming growth factor beta (TGFβ) alone or with a combination of 10 ng/ml of TGFβ with 500 nM of EDP-305 or OCA to assess the effects of EDP-305 on liver fibrosis. Key inflammatory and fibrotic genes were analyzed by RT-PCR.

Results

In comparison to OCA, EDP-305 significantly mitigated the inflammatory response associated with NASH. For example, when compared to OCA, EDP-305 significantly ($p<0.05$) decreased expression of c-c chemokine receptor type 2 (CCR2) by 52%. Furthermore, EDP-305 exhibited an even stronger regulatory effect than OCA ($p<0.01$) by decreasing expression of nuclear factor kappa B (NFκB) by 42%, toll-like receptor 2 (TLR2) by 45%, tumor necrosis factor α (TNFα) by 36%, interleukin 8 (IL8) by 37%, colony stimulating factor-1 (CSF-1) by 46%, chemokine (C-C Motif) Receptor 1 (CCR1) by 54%, interleukin 1β (IL1β) by 41%, and interleukin 1 receptor 1 (IL1R1) by 42%. In addition to its anti-inflammatory effects, EDP-305 also displayed more potent anti-fibrotic effects when compared to OCA. For example, when compared to OCA, EDP-305 significantly ($p<0.05$) decreased expression of α-smooth muscle actin (α-SMA) by 68%, collagen type 1 α2 (COL1A2) by 42%, and collagen type 3 α1 (COL3A1) by 57%. Moreover, when compared to OCA, EDP-305 even further decreased expression ($p<0.01$) of metallopeptidase inhibitor 1 (TIMP1) by 80% and metallopeptidase inhibitor 2 (TIMP2) by 65%, which are critical genes involved in the progression of liver fibrosis.



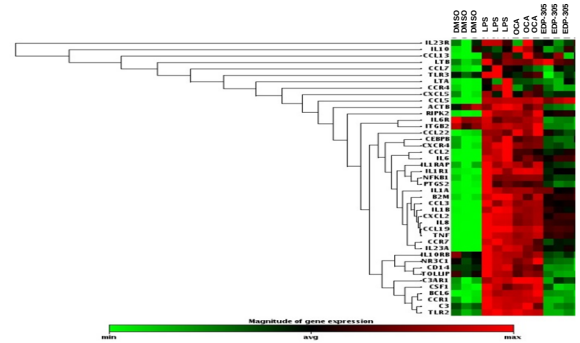


Figure 1. Heat map showing the effects of EDP-305 and OCA on expression of genes involved in inflammation and fibrosis. Relative expression of genes, normalized to control, was calculated from delta C_T values. A, EDP-305 down-regulated inflammatory response genes. B, EDP-305 down-regulated fibrosis genes.

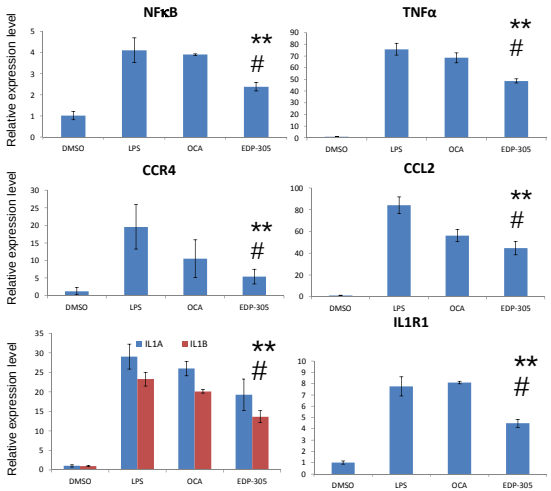


Figure 2. Activation of FXR signaling with EDP-305 significantly inhibited expression of key inflammatory genes *in vitro*. THP1 cells were treated with LPS (50 ng/ml) alone or in combination with OCA (50 nM) or EDP-305 (50 nM) for 18 hours (n=3 for each treatment). # P<0.05 compared to LPS; ** P<0.05 compared to OCA.

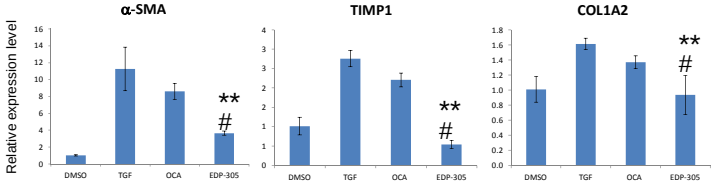


Figure 3. Activation of FXR signaling with EDP-305 significantly inhibited expression of key inflammatory genes *in vitro*. HSCs cells were treated with TGF (10 ng/ml) alone or in combination with OCA (0.5 μM) or EDP-305 (0.5 μM) for 18 hours (n=3 for each treatment). # P<0.05 compared to LPS; ** P<0.05 compared to OCA.

Table 1. Regulation of key genes by EDP-305 and OCA

Genes	EDP-305	OCA
Inflammation		
Nuclear factor kappa B (NF-kB) ↓	+++	+
Toll-like receptor 2 (TLR2) ↓	++++	+
Tumor necrosis factor α (TNF α) ↓	++++	+
Interleukin 8 (IL8) ↓	++++	+
Interleukin 1α (IL1α) ↓	+++	+
Interleukin 1(IL1β) ↓	++++	+
Interleukin 1 receptor 1 (IL1R1) ↓	++++	+
C-C motif ligand 2 (CCL2) ↓	+++	+
C-C chemokine receptor type 1 (CCR1) ↓	+++	+
C-C chemokine receptor type 2 (CCR2) ↓	+++	+
C-C chemokine receptor type 4 (CCR4) ↓	+++	+
Fibrosis		
Alpha smooth muscle actin (α-SMA) ↓	++	+
Metallopeptidase Inhibitor 1 (TIMP1) ↓	++++	+
Metallopeptidase Inhibitor 2 (TIMP2) ↓	++++	+
Platelet derived growth factor a (PDGFA) ↓	++	+
Platelet derived growth factor b (PDGFB) ↓	++	+
Collagen type1 alpha 2 (COL1A2) ↓	+++	+
Collagen type 3 alpha 1 (COL3A1) ↓	+++	+
CCAAT/enhancer-binding protein beta (CEBPB) ↓	+++	+

"+": Biological effects observed
"++": EDP-305 efficacy is better than OCA but does not reach statistical significance;
"+++": EDP-305 efficacy is significantly better than OCA with P<0.05;
"++++":EDP-305 efficacy is significantly better than OCA with P<0.01.

Conclusions

EDP-305 is more potent than OCA in reducing expression of key inflammatory and fibrotic genes *in vitro*, thus holding the potential to mitigate the inflammatory and fibrotic responses associated with NASH.

Acknowledgements

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References

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2. Hepatic inflammation and fibrosis: Functional links and key pathways. Hepatology. 2015 Mar;61(3):1066-79. doi: 10.1002/hep.27332. Epub 2015 Jan 28.