EDP-305 Favorably Regulates Lipoprotein Mechanism *In Vitro and In Vivo*

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Introduction

The Farnesoid X Receptor (FXR) has emerged as an attractive target for the treatment of non-alcoholic steatohepatitis (NASH). EDP-305, a selective and potent small molecule FXR agonist, is in clinical development for the treatment of NASH and primary biliary cholangitis. We previously demonstrated that EDP-305 is more potent than obeticholic acid (OCA) in activating downstream FXR target genes involved in bile acid metabolism. In this preclinical study, we further characterized the metabolic effects on lipoprotein metabolism observed with EDP-305 treatment when compared to OCA.

Materials and Methods

Cell Culture and Treatments. To examine the mechanisms of EDP-305 and OCA independent of drug potency, treatments were conducted near their respective EC_{50} concentrations (OCA @ 500nM; EDP-305 @ 50nM) in human hepatocytes (hepatoma: Huh7.5/HepG2, differentiated hepatic cells: HepaRG, and InSphero 3D liver microtissues).

Animal studies. C57B6/J mice were treated daily by oral gavage for 5 days with EDP-305 (30mpk) or OCA (100mpk).

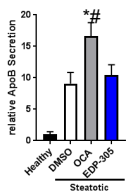
Protein. Hepatocyte cell lysates (15-30 μg protein) were separated by 8% SDS-PAGE, transferred to nitrocellulose membrane, blocked with 5% nonfat dry milk and probed with antibodies against LDLR (Abcam) or SR-B1 (Novus Biologicals).

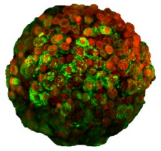
Immunofluorescence. Treated cell monolayers were fixed in 4% paraformaldehyde, rinsed with PBS, blocked with 3% BSA in PBS, and incubated with primary antibodies against LDLR (Abcam) or SR-B1 (Novus Biologicals), followed by incubation with goat anti-rabbit Alexa488. Images were taken with 100x objective (EVOS FL Microscope).

LDL/HDL Uptake. HepG2 cells pretreated with FXR agonists were incubated with fluorescent BODIPY-labeled LDL-C (Invitrogen) or fluorescent HDL-C particles (Biovision) for 2h prior to measurement.

ApoB Secretion. ApoB, a substantial component of non-HDL cholesterol, was measured in 3D human liver microtissues (InSphero) by homogeneous time resolved fluorescence (HTRF, Cisbio) under steatotic conditions.

EDP-305 Does Not Increase ApoB Secretion Under Steatotic Condition





3D Liver Microtissue
(Image from Insphero.com)

Figure 1. ApoB secretion by 3D human liver microtissues treated with EDP-305 (50nM) or OCA (500nM) for 10 days under steatotic conditions (palmitic/oleic acid). *p<0.05 relative to DMSO Steatotic; #p<0.05 relative to EDP-305.

EDP-305 Increases LDLR mRNA and Protein

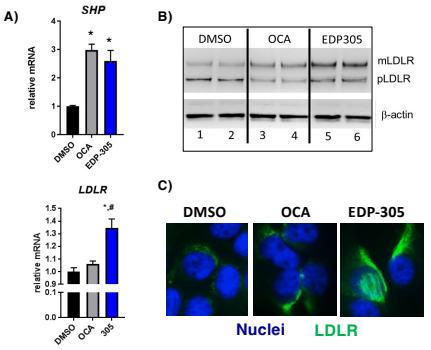


Figure 2. Huh7.5 cells treated with EDP-305 (50nM) or OCA (500nM)for 32 hours under basal conditions (1%FBS). A) Relative *SHP* and *LDLR* mRNA expression. B) Membrane LDLR western blot. C) LDLR immunofluorescence. *p<0.05 relative to DMSO; #p<0.05 relative to OCA.

EDP-305 Increases LDL-C Uptake

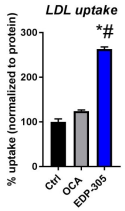


Figure 3. LDL-Cholesterol (LDL-C) uptake in HepG2 cells treated with EDP-305 (50nM) or OCA (500nM) under basal conditions. *p<0.05 relative to control (Ctrl); #p<0.05 relative to OCA.

LDLR Under Steatotic Conditions Is Not Decreased by EDP-305

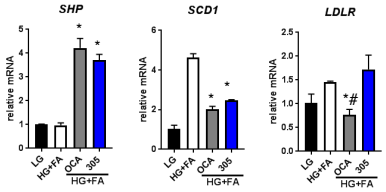


Figure 4. Relative *Cyp7a1*, *SHP* and *LDLR* mRNA expression in HepaRG cells treated with EDP-305 (50nM) or OCA (500nM) under high glucose (HG, 25mM) and fatty acids (FA, 400uM). *p<0.05 relative to HG+FA; #p<0.05 relative to EDP-305; LG=low glucose (0.5mM)

EDP-305 Does Not Increase SRB1

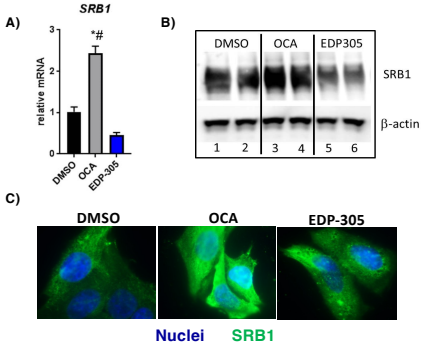


Figure 5. Hepatomas treated with EDP-305 (50nM) or OCA (500nM) for 32 hours under basal conditions (1%FBS). A) Relative *SRB1* mRNA expression (Huh7.5). B) SRB1 western blot (Huh7.5) C) SRB1 Immunofluorescence (HepG2). *p<0.05 relative to DMSO; #p<0.05 relative to EDP-305.

EDP-305 Does Not Increase HDL-C Uptake

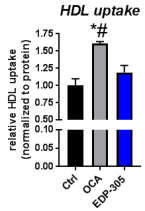


Figure 6. HDL-Cholesterol (HDL-C uptake) in HepG2 cells treated with EDP-305 (50nM) or OCA (500nM) under basal conditions. *p<0.05 relative to control (Ctrl); #p<0.05 relative to EDP-305.

SRB1 Under Steatotic Conditions Is Not Affected by EDP-305

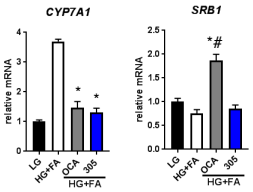


Figure 7. Relative *SRB1* mRNA expression in HepaRG cells treated with EDP-305 (50nM) or OCA (500nM) under high glucose (HG, 25mM) and fatty acids (FA, 400uM). *p<0.05 relative to HG+FA; #p<0.05 relative to EDP-305; LG=low glucose (0.5mM).

EDP-305 Increases LDLR and Does Not Increase SRB1 mRNA *In Vivo*

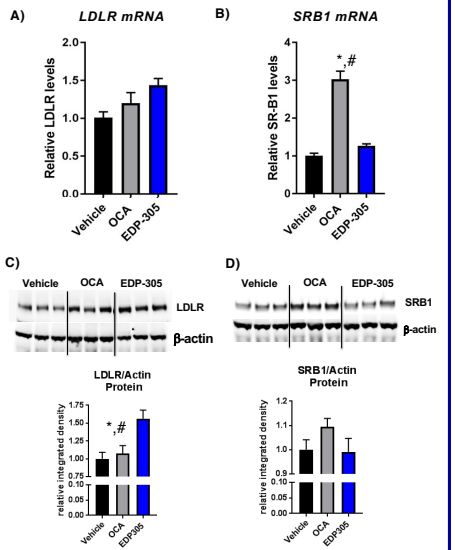


Figure 8. A and B) Relative LDLR and SRB1 mRNA expression in C57Bl6 mice treated with compounds. **C and D)** Western blot of LDLR and SRB1 in liver tissue from treated C57Bl6 mice.
*p<0.05 relative to DMSO; #p<0.05 relative to EDP-305.

Conclusions

- Under basal conditions, EDP-305 favors a positive lipoprotein profile *in vitro* by increasing LDL-C uptake via up-regulation of LDLR, while HDL-C uptake remains unaffected, consistent with a lack of effect on SRB1.
- Under steatotic conditions, EDP-305 maintains a positive effect on lipoprotein metabolism by not altering LDLR or SRB1 expression, without increasing ApoB secretion.
- In summary, EDP-305 is an FXR agonist with a different regulatory effect on lipoprotein metabolism, making EDP-305 attractive for further investigation in NASH treatment.

Acknowledgement

- We thank Ruichao Shen for synthesizing the compounds.