MGH/HST Athinoula A. Martinos Center for Biomedical Imaging

MASSACHUSETTS GENERAL HOSPITAL | HST Harvard-MIT Health Sciences & Technology

The Novel FXR Agonist, EDP-305, Reduces Fibrosis Progression in Rodent Models of Primary Biliary Cholangitis and Non-alcoholic Steatohepatitis

Derek J. Erstad, Christian T. Farrar, Sarani Ghoshal, Lan Wei, Ji-Kyung Choi, Diego Dos Santos Ferreira, Ricard Masia, Nicholas Rotile, Phillip A. Waghorn, Yang Li, Mary Chau, Kenneth K. Tanabe, Yat Sun Or, Peter Caravan, Lijuan Jiang, and Bryan C. Fuchs

MASSACHUSETTS GENERAL HOSPITAL CANCER CENTER

HARVARD MEDICAL SCHOOL

BACKGROUND

- Non-alcoholic steatohepatitis (NASH) is caused by abnormal lipid accumulation in hepatocytes, resulting in liver inflammation and eventual fibrosis
- NASH affects 5% of Americans, with rising incidence due to increasing rates of obesity; currently there are no effective treatments for this condition
- Primary Biliary Cholangitis (PBC) is a autoimmune disease of the liver that results in destruction of the bile ducts leading to cholestasis, inflammation, and fibrosis
- The FXR pathway, which regulates lipid metabolism and bile acid production, represents a therapeutic target for the treatment of both NASH and PBC
- Enanta Pharmaceuticals, Inc. has developed a novel FXR agonist, EDP-305, which we tested here in models of NASH and PBC

RESULTS

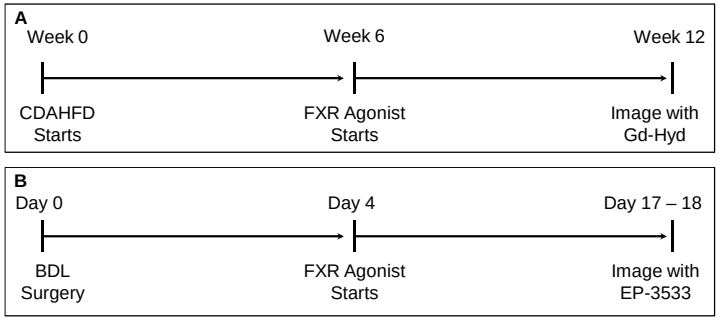
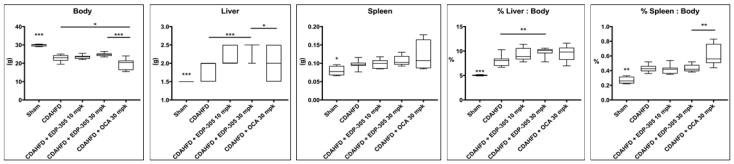


Figure 1. Experimental Design Using Two Different Rodent Models of Fibrosis. (A) Choline-deficient high fat diet (CDAHFD). Concomitant dietary removal of choline with 60 kcal % fat results in steatohepatitis and liver fibrosis within 6 weeks of treatment. (B) Bile duct ligation (BDL) results in obstructive jaundice and hepatic necrosis and fibrosis within 2 weeks. EDP-305, a novel FXR agonist, was given at low (10 mg/kg) and high (30 mg/kg) doses for each model. Obeticholic acid (OCA) was given to CDAHFD mice at a dose of 30 mg/kg. In BDL rats, OCA was given at both 10 mg/kg and 30 mg/kg; the higher dose was lethal. N = 8 for all treatment groups except sham (N = 4).

A. CDAHFD: Body, Liver, and Spleen Weights



B. BDL: Body, Liver, and Spleen Weights

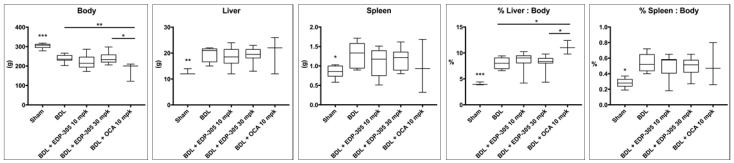


Figure 2. Study Animal Body, Liver, and Spleen Weights. Rodents were sacrificed immediately after imaging. Gross measurements including body, liver, and spleen weights were documented for (A) CDAHFD mice and (B) BDL rats. In both models, rodents treated with OCA were smaller and had less gonadal and visceral fat (*p < 0.05). OCA-treated livers weighed less, and had less fatty accumulation with more fibrosis compared to positive controls. Livers treated with EDP-305 weighed more. The ratio of spleen to body weight was increased in the OCA groups, indicative of advanced liver fibrosis and portal hypertension. * < 0.05, ** < 0.01, *** < 0.001, **** < 0.0001.

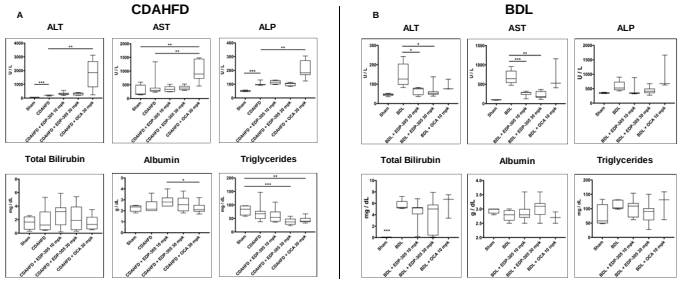


Figure 3. Serum Liver Function Tests and Lipids. (A) CDAHFD mice treated with OCA had significantly elevated ALT, AST, and ALP. There was no difference to bilirubin level between groups. CDAHFD mice treated with EDP-305 had significantly greater albumin than the OCA group. Both EDP-305 at 30 mpk and OCA at 30 mpk were associated with significantly reduced triglycerides compared to controls. (B) EDP-305 at both doses and OCA were associated with reduced AST and ALT compared to BDL positive control. All BDL treatment groups had elevated bilirubin compared to sham.

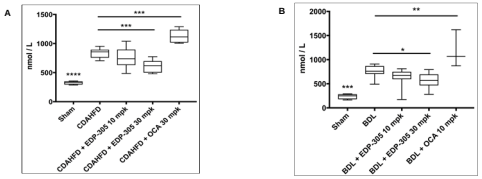


Figure 4. Hydroxyproline Quantification as a Surrogate of Collagen Deposition and Marker of Fibrosis. (A) CDAHFD mice treated with OCA had more hydroxyproline than positive control (CDAHFD), while CDAHFD mice treated with EDP-305 30 mpk had less. (B) Rats treated with OCA had significantly greater hydroxyproline levels compared to positive control (BDL), while rats treated with EDP-305 30 mpk had less.

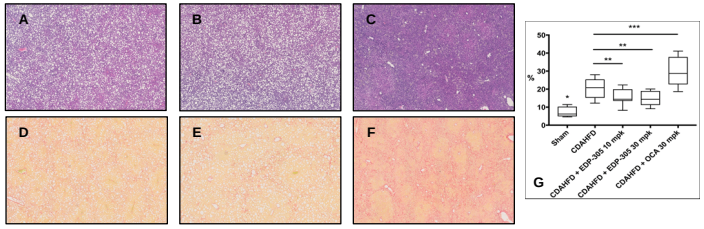


Figure 5. Histologic Sections of CDAHFD Treated Mice. H&E (A-C) and Sirius Red (D-F) histology of CDAHFD mice without drug (A, D), treated with EDP-305 30 mpk (B, E), and OCA 30 mpk (C, F). EDP-305 treated mice have less fibrosis compared to CDAHFD and OCA groups. OCA-treated mice have less steatosis but markedly more fibrosis compared to other groups. (G) Collagen Proportional Area. Determination of collagen surface area coverage as a surrogate measure of liver fibrosis via analysis of Sirius Red staining using ImageJ open source software. Similar to hydroxyproline, OCA treatment had significantly worse fibrosis, whereas the EDP-305 groups had less fibrosis than positive control.

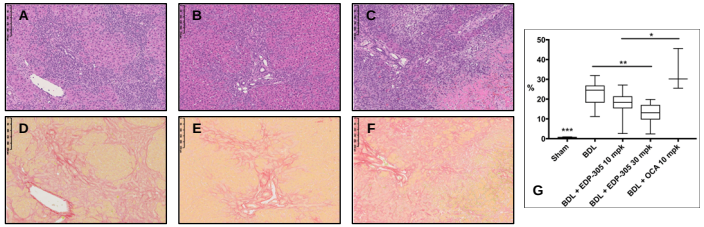


Figure 6. Histologic Sections of BDL Treated Rats. H&E (A-C) and Sirius Red (D-F) histology of BDL rats without drug (A, D), treated with EDP-305 30 mpk (B, E), and OCA 10 mpk (C, F). EDP-305-treated rats have less fibrosis than BDL only and OCA groups. OCA-treated mice have markedly more fibrosis compared to other groups. (G). CPA for BDL rats. Similar to hydroxyproline, OCA treatment had significantly worse fibrosis, whereas the EDP-305 groups had less fibrosis than positive control.

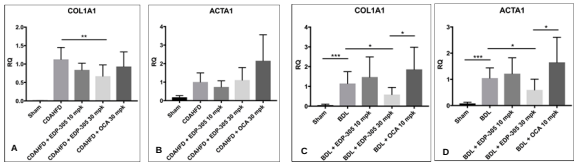


Figure 8. Expression of Fibrogenic Genes. PCR analysis of liver RNA. Collagen-1 expression is significantly lower in animals treated with EDP-305 30 mpk compared to positive controls in (A) CDAHFD and (C) BDL models. Livers treated with OCA have markedly higher collagen and SMA expression relative to the EDP-305 30 mpk group in the BDL model.

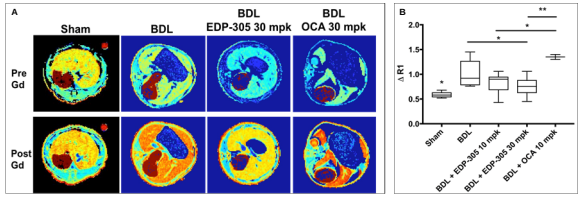


Figure 9. Collagen-targeted MRI in BDL Rats. (A) Rats that underwent a sham procedure or BDL were imaged 17-19 days post surgery immediately prior to (pre Gd) or 45 minutes post (post Gd) injection of the collagen-targeted peptide probe EP-3533. (B) Longitudinal relaxation rate (R1) was measured immediately prior to and 45 minutes post injection of EP-3533. The change in R1 (ΔR1) was assessed as a measure of fibrosis. EDP-305 30 mpk decreases collagen-targeted molecular MRI.

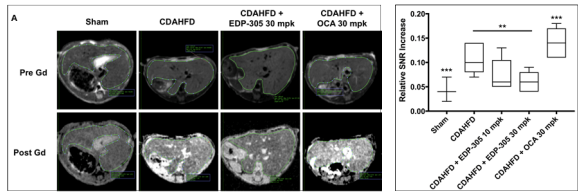


Figure 10. Collagen crosslinking-targeted MRI in CDAHFD Mice. (A) Mice given regular chow (sham) or choline-deficient high fat diet were imaged after 12 weeks of diet and 6 weeks of drug therapy. Images were captured prior to (pre Gd) and 25 minutes post (post Gd) injection of the collagen crosslinking-targeted peptide probe Gd-Hyd. (B) T1 intensity was measured immediately prior to and 25 minutes post injection of Gd-Hyd. The relative increase in signal to noise ratio (SNR) was calculated as {SNR(Post Gd-Hyd) - SNR(Pre Gd-Hyd)}/SNR(Pre Gd-Hyd). EDP-305 30 mpk decreases collagen crosslinking-targeted molecular MRI.

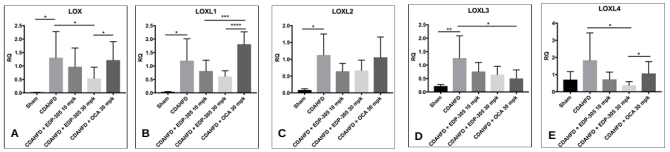


Figure 11. Expression of Lysyl Oxidase Isoforms in CDAHFD mice. PCR analysis of liver RNA. Lysyl oxidase (LOX) cross-links collagen via aldehyde modification of ε amino groups on lysine residues. The Gd-Hyd probe binds to these modified groups. There was a dose-dependent reduction in lysyl oxidase expression with EDP-305 treatment. EDP-305 30 mpk was associated with significantly reduced expression of (A) LOX (F) LOXL4. OCA was associated with increased (B) LOXL1 expression compared to EDP-305.

CONCLUSIONS

- EDP-305 reduced liver fibrosis in CDAHFD and BDL rodent models of fibrosis
- OCA unexpectedly increased fibrosis in both rodent models; animals in this model were smaller, sicker and had a higher mortality rate
- EP-3533 and Gd-Hyd signal intensity correlated with histologic, genetic, and biochemical markers of fibrosis; both probes have short half-lives with renal excretion and should be safe for translation into humans where they may predict response to anti-fibrotic therapy better than traditional biopsy

ACKNOWLEDGEMENTS

This work was supported by grants from the National Institute of Diabetes and Digestive and Kidney Diseases (DK104956 (B.C.F.)), the National Institute of Biomedical Imaging and Bioengineering (EB009062 (P.C.)), and a Research Award from Enanta Pharmaceuticals.