Discovery and Characterization of EDP-721, a Novel Hepatitis B Virus RNA Destabilizer

Michael Vaine, Tessa Cressey, Anand Balakrishnan, Victor Dellisola, Shu-Fan Chou, Joe Panarese, Dexter Davis, Nate Kenton, Samuel Bartlett, Yat Sun Or, Bryan Goodwin, Kai Lin

Enanta Pharmaceuticals, Inc., Watertown, Massachusetts 02472, USA

ENANTA Pharmaceuticals

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BACKGROUND

Available therapies for chronic hepatitis B virus (HBV) infection are effective at reducing both the circulating viral load and the risk for developing hepatocellular carcinoma. However, these therapies rarely result in viral clearance and require lifelong treatment. One barrier to a functional cure for chronic HBV (CHB) is the abundance of circulating surface antigen (HBsAg), which is believed to play an immunosuppressive role. Here we report the discovery of EDP-721, a potent and selective inhibitor of the non-canonical poly(A) RNA polymerase-associated domain containing proteins 5 and 7 (PAPD5 and PAPD7). These host factors play a central role in the post-transcriptional stabilization of HBV RNA and their inhibition results in the destabilization of viral transcripts leading to reduced production of viral proteins, including HBsAg. We show that EDP-721 is effective at reducing HBV RNA and protein in both *in vitro* and *in vivo* models of HBV infection.

METHODS

The activity and selectivity of EDP-721 against the cellular poly(A) polymerases (PAP) PAPD5, PAPD7, PAPα, PAPD1, and PAPD4 was determined biochemically in an enzyme inhibition assay that monitors production of pyrophosphate as a marker for ATP incorporation into an A_{15} or $(CUGC)_5$ RNA substrate. Host RNA effects of EDP-721 treatment were evaluated by RNA-Seq analysis in uninfected primary human hepatocytes. Antiviral activity was assessed in the AD38 and 2.2.15 cell lines, which express HBV in an inducible or stable manner, as well as in cells permissive to HBV infection, including HepG2-NTCP and primary human hepatocytes. Activities against 8 different HBV genotypes (A-H) were determined in transiently transfected HepG2 cells. EDP-721 combinations with nucleos(t)ide reverse transcriptase inhibitors (NRTIs) and the core inhibitor EDP-514 were evaluated *in vitro* in 2.2.15 cells.
In vivo efficacy studies were conducted in C57BL/6 mice infected with an adeno-associated virus (AAV) vector delivering a genotype D HBV genome. HBsAg and HBeAg were quantified with commercially available reagents.

RESULTS

EDP-721 is a Selective Inhibitor of PAPD5 and PAPD7

Enzyme	PAPD5	PAPD7	PAPα	PAPD1	PAPD4
EDP-721 IC_{50} (nM)	3.1	28.1	>10,000	>10,000	>10,000
RG7834 IC_{50} (nM)	91	1237	>10,000	>10,000	>10,000

Table 1. EDP-721 Poly(A) polymerase inhibition selectivity.
Enzyme inhibition was determined by co-incubation of compound with ATP and an RNA substrate and monitoring pyrophosphate production using an enzyme linked luminescent assay.

EDP-721 is an RNA Competitive Inhibitor of PAPD5/7

Enzyme	EDP-721 vs RNA		EDP-721 vs ATP		K_D (nM)
	Kic (nM)	Mechanism	Kiu (nM)	Mechanism	
PAPD5	2.4	Competitive	3.4	Uncompetitive	21
PAPD7	10.0	Competitive	28	Uncompetitive	ND

Table 2. Kinetic parameters for inhibition of PAPD5 and PAPD7 by EDP-721.
Mechanism of inhibition was determined by monitoring EDP-721 mediated inhibition in the presence of increasing amounts of RNA or ATP and curve fitting with competitive or uncompetitive models. K_D for EDP-721 binding in presence of AMPCPP::Mn^{+2} was determined by ITC.

Minimal Effects of EDP-721 on Host Transcriptome

	24 Hour EDP-721 Treatment (nM)			72 Hour EDP-721 Treatment (nM)		
	1000 nM	100 nM	10 nM	1000 nM	100 nM	10 nM
CRP	-2.43	-2.51	-2.36	-2.14	-2.36	-2.31
ALDH3A1				-1.51	-1.76	-1.47
BCYRN1		1.02		1.09		1.13
CHI3L1				-1.33	-1.53	-1.79
H19	-1.69	-1.08	-1.29			
MYO1D	-1.05	-1.09			-1.03	
NDUFA4L2	-2.38	-2.57	-2.28			
PANX2	-1.22	-1.3	-1.2	-1.22	-1.14	-1.24
RHCG	-1.45	-1.27	-1.21			
TOMM34	-1.22	-1.24	-1.17			

Table 3. RNA-Seq analysis of EDP-721 treated primary human hepatocytes.
Uninfected primary human hepatocytes were treated with the indicated concentration of EDP-721 for 24 or 72 hours prior to harvest and RNA-Seq analysis. Genes identified with significant differential expression in at least three treatment conditions are listed above. Values indicate log_2 fold changes from paired vehicle treated samples.

EDP-721 Destabilizes HBV RNA

- EDP-721 destabilizes HBV RNA by reducing mRNA poly(A) tail maintenance

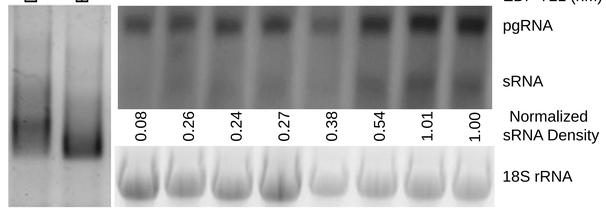


Figure 1. Measurement of HBV poly(A) tail length and RNA levels
HBV poly(A) tail length was monitored by PCR assay in AD38 cells. HBV RNA levels in EDP-721 treated 2.2.15 cells were determined by Northern blot

EDP-721 Blocks HBsAg and HBcAg Synthesis *in vitro*

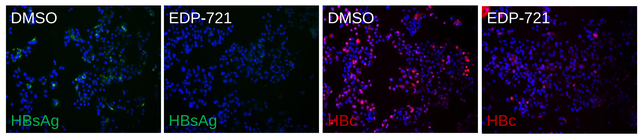


Figure 2. Immunofluorescent staining of AD38 cells demonstrates EDP-721 induced reductions of HBsAg & HBcAg

Cell Line	EDP-721 HBsAg EC_{50} (nM)	RG7834 HBsAg EC_{50} (nM)
2.2.15	0.17 ± 0.04	2.3 ±1.41
HepG2-NTCP	0.57	5.9 ± 0.85
Primary Human Hepatocytes	0.4 ± 0.19	8.4 ± 3.36

Table 4. EDP-721 anti-HBsAg activity in stable and infectable cell lines.
HepG2-NTCP and primary human hepatocytes were infected with AD38 sourced HBV. At three days post infection, or at the time of plating for 2.2.15 cells, EDP-721 was added for five days.

EDP-721 is a Pangenotypic HBsAg Inhibitor

Genotype	A	B	C	D	E	F	G	H
EC_{50} [nM] (Mean ± SD)	0.49 ± 0.33	0.11 ± 0.02	0.19 ± 0.13	0.34 ± 0.15	0.32 ± 0.23	0.66 ± 0.08	0.23 ± 0.14	0.55 ± 0.44

Table 5. EDP-721 reduces HBsAg levels in HBV genotypes A-H.
Representative members of genotypes A-H HBV DNA genomes were synthesized *in vitro*, transfected in HepG2 cells to allow viral replication, and then tested for susceptibility to EDP-721.

Serum Protein Binding Effect

% Human Serum	HBV DNA EC_{50} (nM)	EC_{50} Fold Shift	HBsAg EC_{50} (nM)	EC_{50} Fold Shift
0%	0.09 ± 0.03	-	0.23 ± 0.03	-
10%	0.27 ± 0.02	3	1.04 ± 0.36	4.5
20%	0.35 ± 0.05	3.9	0.93 ± 0.4	4.0
40%	0.55 ± 0.17	6.1	2.07 ± 0.28	9.0

Table 6. Effect of human serum on the anti-HBV activity of EDP-721.
EDP-721 activity was determined *in vitro* in 2.2.15 cells in assay media containing 5% fetal bovine serum, or assay media supplemented with 10%, 20% or 40% normal human serum.

EDP-721 Displays Synergy with Other HBV Antivirals

- Combinations of EDP-721 with NRTIs (entecavir and tenofovir) or a class II HBV core inhibitor (EDP-514) lead to additive to synergistic antiviral effect *in vitro.*

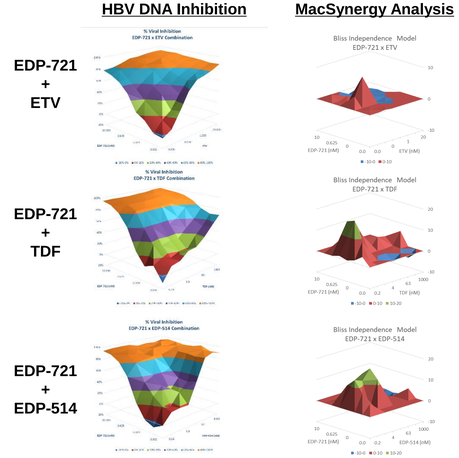


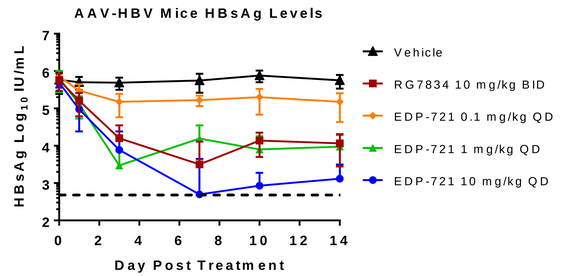
Figure 3. HBV inhibition and MacSynergy (Bliss Independence model) analysis.

Compounds	Combination Index Values at EC_{50}	EC_{75}	EC_{90}
EDP-721 + ETV	0.18	0.44	0.87
EDP-721 + TDF	0.14	0.59	0.87
EDP-721 + EDP-514	0.30	0.35	0.88

Table 7. Analysis of the combinations using Lowe Additivity model (CalcuSyn).
2.2.15 cells were treated with EDP-721, entecavir (ETV), tenofovir disoproxil fumarate (TDF) and EDP-514 at various concentrations either alone or in combination for 5 days. The inhibition of HBV DNA in the cells was determined by qPCR. The data were analyzed for antagonistic, additive, or synergistic effects using two different mathematic models, Bliss Independence model (MacSynergy) and Lowe Additivity model (CalcuSyn).

EDP-721 is Efficacious in an AAV-HBV Mouse Model

- Rapid declines in HBsAg & HBeAg observed within 24 hours of EDP-721 treatment
- HBsAg reductions of 0.67, 1.83 and 2.57-log_{10} observed at day 14 with oral EDP-721 treatment at 0.1, 1, and 10 mg/kg QD respectively
- EDP-721 treatment led to a maximum reduction in circulating HBeAg of 0.84 log_{10} at day 14



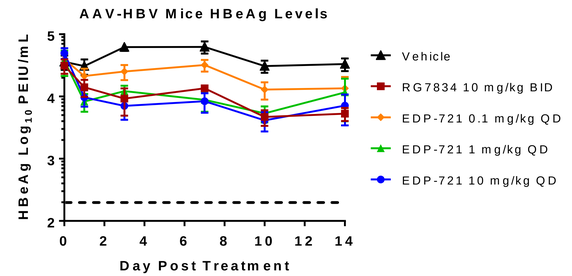


Figure 4. Inhibition of HBsAg and HBeAg in an AAV-HBV mouse model
C57BL/6 mice inoculated with an adeno-associated virus (AAV) expressing a GT-D HBV transgene were treated with EDP-721 for 14 days

CONCLUSIONS

- EDP-721 is a potent and selective inhibitor of the non canonical poly(A) polymerases PAPD5 and PAPD7. Mechanistic studies suggest an RNA competitive binding mode.
- EDP-721 treatment results in minimal changes to the host transcriptome in primary human hepatocytes.
- EDP-721 potently inhibits HBsAg production in multiple cell lines.
- EDP-721 also blocks HBcAg and HBeAg synthesis.
- EDP-721 is active against all HBV genotypes tested (A-H).
- Combinations of EDP-721 with NRTIs or a class II core inhibitor results in additive to synergistic antiviral effects *in vitro.*
- Oral administration of EDP-721 demonstrates excellent *in vivo* efficacy with >2.5-log HBsAg reduction in an AAV-HBV mouse model.
- These data support further development of EDP-721 as an oral therapeutic candidate for HBV.

ACKNOWLEDGEMENTS

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