EDP-514, a Novel HBV Core Inhibitor with Potent Antiviral Activity both *In Vitro* and *In Vivo*

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BACKGROUND

Current therapies for hepatitis B virus (HBV) can effectively suppress viral replication but rarely lead to a functional cure, which creates a need for the development of new antiviral drugs with different mechanisms. The HBV core protein plays an essential role in multiple steps of the viral life cycle and has been proposed as a potential therapeutic target. EDP-514 is a novel HBV core inhibitor with a favorable pharmacokinetic and safety profile. Here, we describe the anti-HBV activities of EDP-514 *in vitro* and in a chimeric mouse model with humanized liver.

METHODS

The mechanism of action (MoA) of EDP-514 was characterized using three different methods: (1) *in vitro* HBV capsid assembly assay, (2) size exclusion chromatography (SEC) analysis of capsid formation, and (3) anti-core staining of compound treated cells. The *in vitro* anti-HBV activities at different stages of the viral lifecycle were examined in three HBV expressing stable cell lines (HepG2.2.15, HepAD38 and HepDE19) and HBV infected primary human hepatocytes. Activities against 8 different HBV genotypes (A-H) and multiple resistance mutations in reverse transcriptase (RT) and core protein were determined in transiently transfected HepG2 cells. Combinations with nucleos(t)ide RT inhibitors and other HBV core inhibitors were evaluated *in vitro* using HepAD38 cells. The *in vivo* efficacy of EDP-514 was demonstrated in HBV genotype C-infected PXB mice which were generated by transplantation of urokinase-type plasminogen activator/severe combined immuno-deficiency (uPA/SCID) mice with human hepatocytes.

RESULTS

Mechanism of Action

- EDP-514 is a class II HBV inhibitor, which stimulates the assembly of empty capsids but does not cause core aggregation *in vitro* or in the cells.

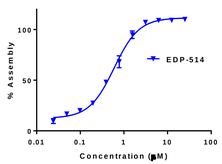


Figure 1. *In vitro* HBV capsid assembly assay.
The assembly domain (N terminal 149AA) of core was labeled with a BODIPY fluorophore, which was quenched upon capsid assembly.

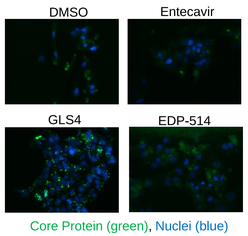


Figure 2. Anti-core IF staining.
HepAD38 cells were treated with 1 μM EDP-514, GLS4 (a class I core inhibitor) or entecavir for 7 days and stained with an anti-core antibody
Core Protein (green), Nuclei (blue)

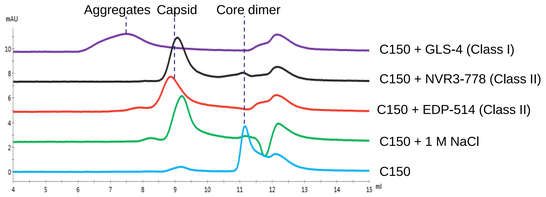


Figure 3. Size exclusion chromatography (SEC) analysis of capsid formation.
Dimeric HBV core protein (C150) (2 μM) was incubated with either 1 M NaCl or 25 μM compounds 2 h and then analyzed using a SRT SEC 1000 column (14.29 mL). MW=16.78 kDa (C150); 33.56 kDa (dimer) 4000 kDa (capsid).

Activity in HBV Stable Cell Lines

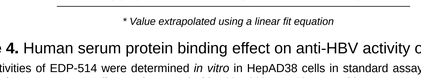
- EDP-514 potently inhibits HBV replication in all three stable cell lines.

Cell Line	HBV DNA EC_{50} (nM)	HBV DNA EC_{90} (nM)	Encapsidated RNA EC_{50} (nM)	HBeAg EC_{50} (nM)
HepAD38	18	63	25	20
HepDE19	27	82	3	34
HepG2 2.2.15	17	85	5	>500

Table 1. Activity of EDP-514 in three HBV expressing stable cell lines.
Cells were treated with EDP-514 for 5 days and then analyzed for intracellular HBV DNA and encapsidated viral RNA. The effect on secreted HBeAg was also determined, which depends on cccDNA production in HepAD39 and HepED19 cells but not in HepG2.2.15 cells.

Activity in Primary Human Hepatocytes

- EDP-514 inhibits not only HBV replication in primary human hepatocytes but also the formation of cccDNA if given early during infection.



	HBsAg EC_{50} (nM)		HBV DNA EC_{50} (nM)	
	d0 Addition	d3 Addition	d0 Addition	d3 Addition
EDP-514	35	>1000	10	6
Entecavir	>1000	>1000	0.25	0.21

Table 2. Activity of EDP-514 in HBV infected primary human hepatocytes.
Cells were treated with EDP-514 or entecavir either at the time of infection (d0 addition) or 3 days post infection (d3 addition). HBsAg, which is transcribed from cccDNA, was measured as a surrogate marker to determine the ability of the compound to prevent the formation of new cccDNA if given early during infection.

Activity against HBV Genotypes

Genotype	A	B	C	D	E	F	G	H
EC_{50} [nM] (Mean ± SD)	16±10	9±5	13±9	13±6	21±15	9±2	11±5	32±25

Table 3. EDP-514 is active against all HBV genotypes tested (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-514.

Serum Protein Binding Effect

% Human Serum	HBV DNA EC_{50} (nM)	EC_{50} Fold Shift
0%	10	1.0
10%	19	1.9
20%	42	4.1
40%	58	5.6
100%	134*	13.1

** Value extrapolated using a linear fit equation*

Table 4. Human serum protein binding effect on anti-HBV activity of EDP-514.
The activities of EDP-514 were determined *in vitro* in HepAD38 cells in standard assay media containing 5% fetal bovine serum, vs. media supplemented with 10%, 20% or 40% normal human serum.

Activity Against RT and Core Mutants

- EDP-514 is fully active against known nucleos(t)ide reverse transcriptase inhibitor (NRTI) resistance mutations.
- Among HBV core mutations previously reported resistant to treatment with other core inhibitors, only T33N and Y118F significantly affect susceptibility to EDP-514.

Mutations in RT	EC_{50} (nM)	Fold Shift
WT	11	-
M204I	52	4.7
M204V+L180M	25	2.3
M204V+L180M+V173L	16	1.5
M204V+L180M+V173L+M250V+I169T	22	2.0
M204V+L180M+V173L+N236T	26	2.4
N236T	25	2.3
N236T+A181T	14	1.3

Table 5. Activity against HBV reverse transcriptase (RT) mutants.

Mutations in Core	EC_{50} (nM)	Fold Shift
WT	13	-
D29G	43	3.3
T33N	2937	226
S106T	8	0.6
T109I	5	0.4
T109M	13	1
Y118F	236	18.2
V124F	56	4.3

Table 6. Activity against HBV core mutants.

HBV genomes containing mutations were synthesized in vitro, transfected into HepG2 cells to produce mutant viruses, and then tested for susceptibility to EDP 514.

Combination with Other HBV Inhibitors

- Combination of EDP-514 with NRTIs (entecavir and tenofovir) or a class I HBV core inhibitor GLS4 lead to additive to synergistic antiviral effect *in vitro.*

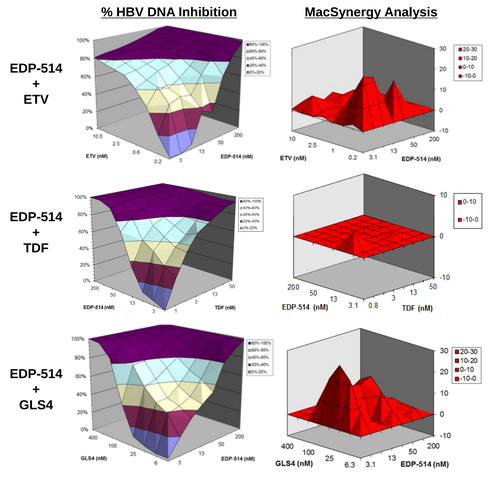


Figure 4. HBV inhibition and MacSynergy (Bliss Independence model) analysis.

Drugs	Combination Index Values at EC_{50}	EC_{75}	EC_{90}
GLS-4 + EDP-514	0.82	0.76	0.77
ETV + EDP-514	0.59	0.47	0.48
TDF + EDP-514	1.06	0.80	0.71

Table 7. Analysis of the combinations using Lowe Additivity model (CalcuSyn).
HepAD38 cells were treated with EDP-514, entecavir (ETV) and tenofovir disoproxil fumarate (TDF) and GLS4 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).

In Vivo Efficacy in Humanized Mouse Model

- Treatment of HBV infected human liver-chimeric mice (PXB mice) with EDP-514 for 12 weeks resulted in a time and dose-dependent viral load reduction.
- The maximum HBV DNA reduction from baseline was 2.99, 3.61, 3.95 and 4.43-log_{10} with EDP-514, given orally at 25, 50, 75 and 100 mg/kg BID, respectively.
- EDP-514 treatment also led to >3-log_{10} reduction in circulating HBV RNA, whereas entecavir had no effect.
- There was a small but significant reduction in HBsAg (0.38-log_{10} at 100 mg/kg) and HBeAg (0.43-log_{10} at 100 mg/kg BID) in mice treated with EDP-514.
- The virus rebounded to baseline after withdrawal of treatment.

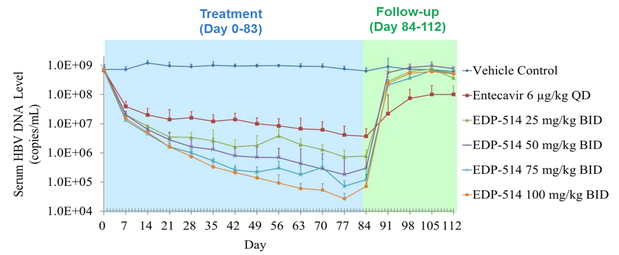


Figure 5. HBV DNA level over time

The human liver-chimeric mice (PXB mice) were generated by transplantation of uPA/SCID mice with human hepatocytes. The mice were infected with an HBV genotype C virus and allowed to proceed for at least 8 weeks to establish chronic infection. 30 HBV-infected PXB mice (n=5 in each group) were treated with vehicle control, entecavir or 4 different doses of EDP-514 for 12 weeks (Day 0-83) and then followed up for 4 more weeks (Day 84-112) after withdrawal of treatment. Serum samples were collected every week to monitor the level of HBV DNA and antigens. Serum samples taken on Day 70 were also analyzed for HBV RNA.

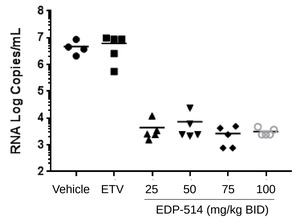


Figure 6. HBV RNA level on Day 70

CONCLUSIONS

- EDP-514 is a novel class II HBV core inhibitor.
- EDP-514 inhibits HBV replication *in vitro* with an EC_{50} of 18, 27 and 17 nM in HepAD38, HepDE19, and HepG2.2.15 cells, respectively.
- EDP-514 prevents the *de novo* formation of new cccDNA in primary human hepatocytes if given early during infection.
- EDP-514 is active against all HBV genotypes tested (A-H).
- Known HBV NRTI resistance mutants are fully susceptible to EDP-514.
- Combinations of EDP-514 with NRTIs or a class I core inhibitor result in additive to synergistic antiviral effect *in vitro.*
- EDP-514 demonstrates excellent *in vivo* efficacy with >4-log viral load reduction in HBV-infected PXB mice.
- These data support further development of EDP-514 as a therapeutic candidate for HBV.

ACKNOWLEDGEMENT

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