THU309

Polymorphic Analysis of Bulevirtide Sequence in preS1 of Large HBsAg Across HBV Genotypes A–H

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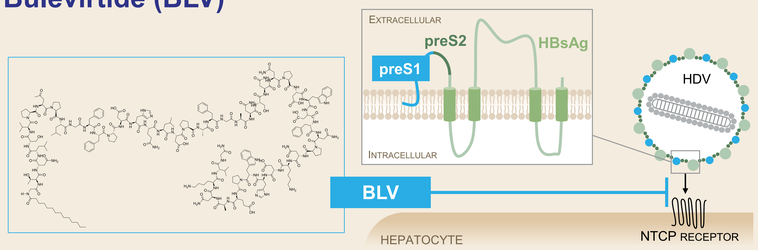
Introduction

- Hepatitis delta virus (HDV) is a hepatotropic, single-stranded RNA virus that utilizes the surface proteins from hepatitis B virus (HBV) for viral entry, dissemination, and assembly, with genotype (GT) 1 being the most common globally
- Both viruses enter hepatocytes through interaction of the preS1 domain of the large hepatitis B surface antigen (L-HBsAg) with the sodium taurocholate cotransporting polypeptide (NTCP) receptor[1,2]
- Crucial for binding within the N-terminal preS1 domain, the sequence 9-NPGLFFP-15 is highly conserved among HBV GT; point mutations at amino acids (aa) L11, G12, and F13 lead to complete loss of infectivity of HBV mutants[3]

Bulevirtide (BLV)



- BLV is a novel 47-aa, N-terminally myristoylated lipopeptide that binds specifically to NTCP and acts as a potent, highly selective entry inhibitor of HDV into hepatocytes
- The BLV sequence was designed based on the consensus sequence of the L-HBsAg preS1 domain of HBV GT A–H

Objective

- To investigate the impact of polymorphisms found in the L-HBsAg preS1 domain from HBV clinical isolates of 8 HBV GT on susceptibility to BLV

Methods

- Polymorphic residues in the BLV sequence region of preS1 were identified using an alignment of 7427 preS1 sequences of HBV GT A–H obtained from clinical isolates and public databases; for GT A–D, a minimum number of 958 (GT A) and up to 2605 (GT C) sequences were analyzed
- Consensus (representative) L-HBsAg sequences for each HBV GT and a total of 24 HBsAg constructs that represent the most frequent polymorphic changes observed in patient sequences were constructed: 2 for GT A, 10 for GT B, 10 for GT C, and 2 for GT D
- For the more prevalent HBV GT A–D, residues prevalent at frequencies >5% compared with the BLV sequence were also identified and tested for BLV susceptibility
- HDV GT 1 viruses containing representative sequences or these polymorphisms in L-HBsAg were generated by transient transfection and analyzed phenotypically for their susceptibility to BLV in an infectious assay using primary human hepatocytes

Results

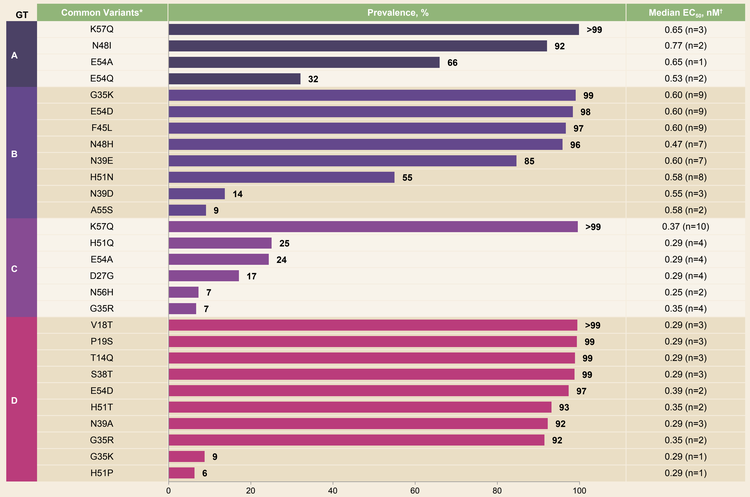
Figure 1. BLV Activity Against HDV GT 1 Enveloped With Representative preS1 Sequences From HBV GT A–H*

	Mean EC_{50}, nM†	aa Position‡ 1 5 10 15 20 25 30 35 40 45 50 55	aa Changes
BLV	—	G T N L S V P N P L G F F P D H Q L D P A F G A N S N N P D W D F N P N K D H W P E A N K V G	—
GT A	0.65	V I A Q	4
GT B	0.60	K E L Y N D	6
GT C	0.46	Q	1
GT D	0.49	Q T S R T A T D	8
GT E	0.85	K H T T R T R H T	9
GT F	0.28	Q L R S S T S M	8
GT G	0.57	K T S L R T K P	8
GT H	0.45	Q L R S S T N M	8

*pLX304-HB2.7-gtA–H; †Mean of 2 replicate values; ‡Numbering corresponds to positions of GT A X02763; BLV sequence was used as reference aa for each position. EC_{50}, half maximal effective concentration.

- Polymorphic analyses of HBV sequences from GT A–H revealed a high degree of conservation of the 47-aa peptide corresponding to the BLV sequence, with a remarkable conservation of the sequence from positions 9-NPGLFFP-15, which is crucial for binding to NTCP
- No consistent polymorphisms in the preS1 BLV region were found across HBV GT A–H
- Compared with the consensus sequence for the preS1 region, only 1 aa residue was found to be variable with a frequency >5% for HBV GT A; GT B, C, and D had 2, 5, and 2 aa above the 5% cutoff, respectively (data not shown)
- Analysis of representative preS1 sequences for the most common HBV GT (A–D) revealed that GT C was the closest to the BLV sequence with only 1 aa change (K57Q); GT A and B had 4 and 6 aa changes, respectively, and GT D had 8 (Figure 1)

Figure 2. Polymorphic BLV Sequence Positions Represented at Frequencies >5% Within preS1 of HBV GT A–D Compared With BLV Sequence



*Numbering corresponds to positions of GT AX02763; BLV sequence was used as a reference aa for each position; †Median of 2 replicate values; n=number of isolates with those variants tested.

Figure 3. BLV Activity Against Viruses With Most Common aa Variations Found in Clinical Isolates of HBV GT A–D

	Mean EC_{50}, nM*	aa Position 1 5 10 15 20 25 30 35 40 45 50 55	aa Changes
BLV	—	G T N L S V P N P L G F F P D H Q L D P A F G A N S N N P D W D F N P N K D H W P E A N K V G	—
GT A consensus	0.65	I A Q	3
HBVA.1-HDV1	0.17	A L R V Q Q	6
HBVA.2-HDV1	0.88	I Q Q	3
GT B consensus	0.60	K E L H N D	6
HBVB.1-HDV1	0.47	A L R E L H N D	8
HBVB.2-HDV1	0.74	L K D L H T D S	8
HBVB.3-HDV1	0.55	L K D L H N D	7
HBVB.4-HDV1	0.42	K D L H Y D S	7
HBVB.5-HDV1	0.41	K E Q H N D	6
HBVB.6-HDV1	0.93	K E L Y N D	6
HBVB.7-HDV1	0.67	K E L Y N D	6
HBVB.8-HDV1	n/a	K E L H T D	6
HBVB.9-HDV1	0.68	K E L Y N D	6
HBVB.10-HDV1	0.43	K E L H N N	6
GT C consensus	0.46	Q	1
HBVC.1-HDV1	0.47	A L R P Q	5
HBVC.2-HDV1	0.21	A L R P H Q	6
HBVC.3-HDV1	0.71	L Q	3
HBVC.4-HDV1	0.24	A L R P Q	5
HBVC.5-HDV1	0.45	G R Q A Q	5
HBVC.6-HDV1	0.28	G L Q A H Q	6
HBVC.7-HDV1	0.88	L P W Q	4
HBVC.8-HDV1	0.29	G Q A Q	4
HBVC.9-HDV1	n/a	Q Q	2
HBVC.10-HDV1	0.26	G Q A Q	4
GT D consensus	0.49	Q T S R T A T D	8
HBVD.1-HDV1	0.29	Q T S K T A P D	8
HBVD.2-HDV1	0.20	Q T S R T A T N	8

*Mean of 2 replicate values.

- HDV GT 1 viruses containing HBV GT A–H preS1 representative sequences and combinations of multiple substitutions covering the most prevalent GT A–D polymorphisms in clinical isolates were fully susceptible to BLV, with EC_{50} values ranging from 0.28 to 0.85 nM (Figures 1–3)
- No significant differences in EC_{50} values were observed for any of the assayed viruses, suggesting these polymorphisms had no effect on BLV susceptibility

Conclusions

- BLV demonstrated potent activity against HDV harboring the most common polymorphisms in the HDV GT 1 preS1 region corresponding to the BLV sequence from patient isolates across HBV GT A–D, indicating broad-spectrum potential for antiviral activity of BLV for the treatment of patients infected with HDV

References: **1.** Ni Y, et al. Gastroenterology 2014;146:1070-83; **2.** Yan H, et al. eLife 2012;1:e00049; **3.** Engelke M, et al. Hepatology 2006;43:750-60.
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