Inhibition of PAPD5 and PAPD7 by Small-Molecule HBV RNA Destabilizers from DHQ and THP Chemical Series

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ABSTRACT

Noncanonical poly(A) polymerases PAPD5 and PAPD7 (PAPD5/7) stabilize HBV RNA through the viral post-transcriptional regulatory element (PRE) [1-3]. Inhibitors of PAPD5/7 reduce HBV RNA, which in turn leads to suppression of viral replication and viral protein production, including HBsAg [4,5]. In this study, representative PAPD5/7 inhibitors from a tetrahydropyridopyrimidine (THP-1) and a dihydroquinolizinone (DHQ, represented by AB-452 and RG7834) chemical series were evaluated against PAPD4/5/7 enzymes and HBV expressing cells. Biochemical data showed that THP-1, but not AB-452, inhibited PAPD4 enzymatic activities. THP-1 also inhibited PAPD5/7 with similar efficiencies, while AB-452 was more active against PAPD5 when compared to PAPD7. Consistent with the biochemical results, AB-452 was more active against HBV infected PAPD7-knockout (KO) cells when compared to wildtype and PAPD5-KO cells, while THP-1 exhibited similar potencies across these cell lines. Furthermore, AB-452 inhibited HBsAg, HBeAg, HBV DNA and HBV RNA with an EC_{50} that ranged from 1.4 to 4.6 nM in multiple HBV cell models. THP-1 reduced HBsAg and HBeAg with similar potencies but was >10-fold less efficient against HBV RNA and HBV DNA in infected primary human hepatocytes. *In vitro* combination studies of AB-452 with capsid inhibitors or nucleoside analogs showed additivity to moderate synergy. Interestingly, analysis of intracellular HBV RNA revealed that pgRNA was more robustly degraded by AB-452 in the presence of capsid inhibitors, supporting potential combination strategies of HBV RNA destabilizers with capsid inhibitors.

OBJECTIVES

- To evaluate the antiviral activities of AB-452 and THP-1
- To determine PAPD5, PADP7, and PAPD4 enzymatic inhibition by HBV RNA destabilizers from DHQ and THP classes
- To assess combination effect of AB-452, nucleos(t)ide analogs, and HBV capsid inhibitors

RESULTS

Figure 1. AB-452 and THP-1 inhibit multiple steps within the viral life cycle

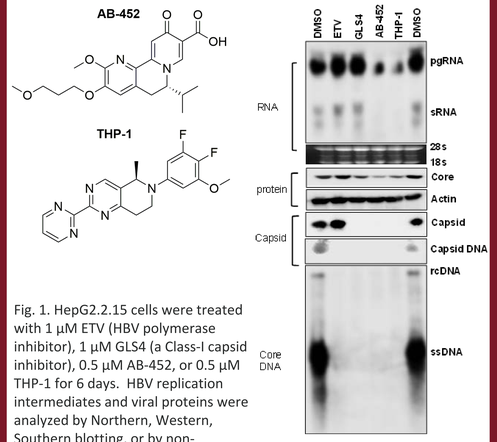


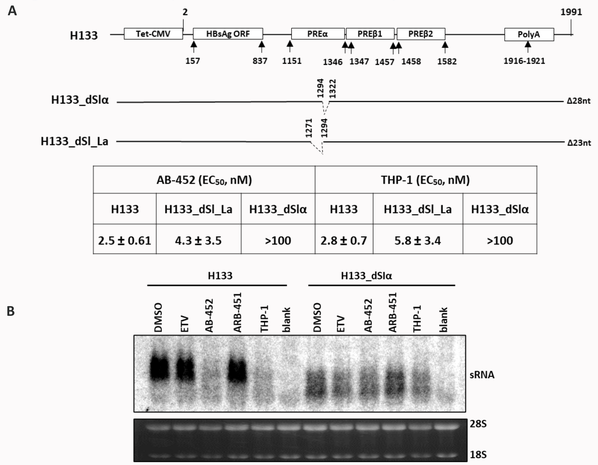
Fig. 1. HepG2.2.15 cells were treated with 1 µM ETV (HBV polymerase inhibitor), 1 µM GLS4 (a Class-I capsid inhibitor), 0.5 µM AB-452, or 0.5 µM THP-1 for 6 days. HBV replication intermediates and viral proteins were analyzed by Northern, Western, Southern blotting, or by non-denaturing particle gel electrophoresis.

Table 1. Anti-HBV effect of AB-452 and THP-1 *in vitro* using different HBV cell models

HBV Cell Models	HBV Markers	AB-452 EC_{50} [nM]	THP-1 EC_{50} [nM]
HepG2.2.15	HBsAg	1.4 ± 0.2	0.83 ± 0.3
PLC/PRF/5	HBsAg	2.3 ± 0.36	3.0 ± 0.12
HBV infected PHHs	HBsAg	3.0 ± 2.1	7.92 ± 4.22
	HBeAg	3.7 ± 1.3	1.77 ± 1.61
	HBV RNA	4.6 ± 5.0	**50.2 ± 17.3**
	HBV DNA	4.2 ± 3.8	**45.9 ± 24.2**

Table 1. *In vitro* potency of THP-1 and AB-452 was determined in several HBV cell models. In PHH, THP-1 and AB-452 displayed similar potencies against HBsAg and HBeAg, but THP-1 appears ~10x weaker against HBV RNA and DNA compared to AB-452.

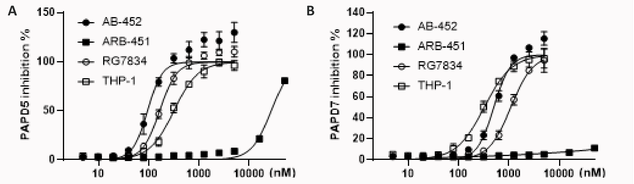
Figure 2. HBV PRE is required for AB-452 and THP-1 activity



AB-452 (EC_{50}, nM)			THP-1 (EC_{50}, nM)		
H133	H133_dSl_La	H133_dSlα	H133	H133_dSl_La	H133_dSlα
2.5 ± 0.61	**4.3 ± 3.5**	**>100**	**2.8 ± 0.7**	**5.8 ± 3.4**	**>100**

Fig. 2. Huh-7 cells were transfected with H133 (wild-type), H133_dLa, or H133_dSLα plasmids and treated with AB-452 or THP-1 for 5 days. (A) Schematic representation of H133, H133_dSLα (SLα deleted, nt 1294 to 1322), and H133_dLα mutants (La binding site deleted, nt 1271 to 1294). Activities against HBsAg production were determined and EC_{50} values summarized. (B) ETV (1 µM), AB-452 (0.1 µM), or ARB-451 (inactive enantiomer of AB-452, 0.1 µM) and THP-1 (0.1 µM) were tested against HBsAg production for 5 days. HBsAg RNA (sRNA) were detected by Northern blot, with ribosomal 18S and 28S RNAs as internal controls.

Figure 3. AB-452 targets PAPD5 selectively, while THP-1 inhibits both PAPD5 and PAPD7 with similar potencies



	AB-452	ARB-451	RG7834	THP-1
PAPD5 IC_{50} [nM]	93.8 ±10.7	27002 ±928	164.2 ±3.6	314.7 ±11.9

	AB-452	ARB-451	RG7834	THP-1
PAPD7 IC_{50} [nM]	498.1 ±50.9	>50,000	1093 ±52.3	325 ±11.8

Fig. 3. Biochemical assay in which the compounds were tested against purified recombinant PAPD5 or PAPD7 enzymes. The readout was the measurement of unused ATPs in the reaction. RG7834 and ARB-451 serve as positive and negative control compounds, respectively.

Table 2. AB-452 activity is reduced in the PAPD5-KO clones but not that of THP-1

Cell lines	Compound	EC_{50} ± SD [nM]	Fold change
Parent cells	AB-452	9.0 ± 4.6	1.0
	THP-1	11.1 ± 7.0	1.0
PAPD7 KO-1	AB-452	10.0 ± 1.0	1.1
	THP-1	16.9 ± 6.1	1.5
PAPD7 KO-2	AB-452	7.1 ± 1.3	0.8
	THP-1	15.4 ± 3.4	1.4
PAPD5 KO-1	AB-452	**56.1 ± 34.0**	**6.3**
	THP-1	6.4 ± 2.4	0.6
PAPD5 KO-2	AB-452	**71.6 ± 31.2**	**8.0**
	THP-1	12.0 ± 4.8	1.1

Table 2. Parental, PAPD5 or PAPD7-knockout (KO) cells were infected with HBsAg expressing-Adenoviruses and treated with AB-452 or THP-1. Fold change is determined by dividing the EC_{50} values from the PAPD5 or PAPD7-KO cells with those from the parental cells for AB-452 or THP-1. Fold change values higher than 3-fold are bolded.

Figure 4. THP-1, but not AB-452, inhibits PAPD4 enzymatic activity

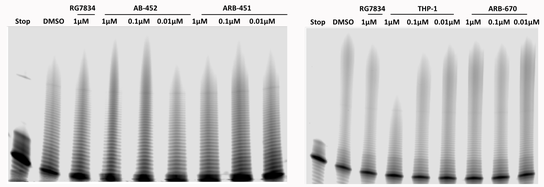


Fig. 4. The polyadenylase activities of PAPD4 were determined in the presence of test compounds. Two days post-transfection of plasmid pCMV-FlagD4 in HEK293 cells, the tagged PAPD4 polypeptides were precipitated with anti-Flag antibody. The precipitated beads, mixed with 1 mM ATP, were incubated with RNA oligonucleotides at 37°C for 20 minutes [6]. ARB-451 and ARB-670 serve as negative controls.

Table 3. *In vitro* combination studies with AB-452 and other HBV inhibitors

Inhibitor A	Inhibitor B	Assay	Conclusion
AB-452	AB-423	bDNA	Moderate synergy
	AB-506		Additive
	TAF		Additive
	ETV		Moderate synergy

Table 3. Treatment of HepG2.2.15 cells with combinations of AB-452, and capsid inhibitors (AB-423, AB-506) or HBV polymerase inhibitors (TAF or ETV) displayed additive to synergistic effects in inhibiting HBV DNA.

Figure 5. AB-452 in combination with GLS-4 enhances pgRNA degradation

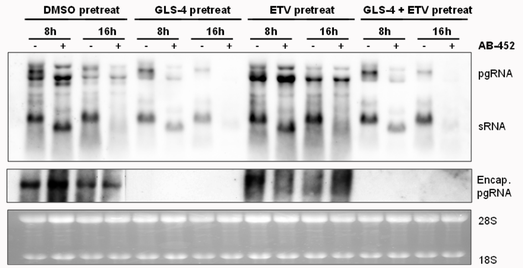


Fig. 5. AB-452 in combination with GLS-4 enhances pgRNA degradation. ETV pretreatment enriched encapsidated pgRNA, likely via blocking reverse transcription of pgRNA, and as expected the enriched encapsidated pgRNA was resistant to AB-452. GLS-4 pretreatment inhibits pgRNA encapsidation, which may increase its susceptibility to AB-452 mediated degradation.

CONCLUSIONS

- AB-452 and THP-1 represent two different chemical series of HBV RNA destabilizers with broad and potent anti-HBV effects.
- Both AB-452 and THP-1 promote viral RNA degradation, resulting in reduced production of HBV proteins, viral DNA replication and virion release.
- Post-transcriptional element (PRE) of HBV sequence is required for the activity of both AB-452 and THP-1 compounds.
- THP-1 inhibits the enzymatic activity of PAPD4, PAPD5, and PAPD7.
- DHQ-1 compounds (RG7834 and AB-452) did not inhibit PAPD4, and inhibited PAPD5 more efficiently compared to PAPD7.
- Consistent with the biochemical results, THP-1 inhibited HBsAg in PAPD5-KO and PAPD7-KO cells with similar efficiencies, while AB-452 was more efficient against the PAPD7-KO cells compared to PAPD5-KO cells.
- AB-452 treatment combined with capsid inhibitor(s) further promoted the degradation of HBV pgRNA, suggesting that further exploration of the combination of an HBV RNA destabilizer and a capsid inhibitor is warranted.

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