Nucleic acid polymers are efficient in blocking hepatitis delta virus entry *in vitro*

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BACKGROUND & AIMS

- Nucleic acid polymers (NAPs) are phosphorothioated oligonucleotides, which exhibit a sequence independent, broad-spectrum antiviral activity.
- NAPs have been previously shown to have antiviral effect against duck hepatitis B virus (DHBV) infection, exerting both entry and post-entry antiviral activities (Noordeen et al., 2013).
- NAPs have been shown to be clinically active against HBV infection, through a rapid reduction of both HBsAg and HBV DNA in the serum of patients.

Ø HBV and hepatitis delta virus (HDV) are assumed to have similar entry mechanisms. We therefore assessed the anti-HDV activity of various NAPs using two *in vitro* cellular infection models.

MATERIALS & METHODS

- The NAPs used in this study are presented in the table.
- Differentiated HepaRG cells, or NTCP- expressing Huh-7 cells, were inoculated with HDV (MOI:100).
- Entry experiments: NAPs were added at the time of viral inoculation.
- Post-entry experiments: NAPS were added 18 h postinoculation.
- Infection was monitored by measuring intracellular HDV RNA levels at day-9 postinoculation

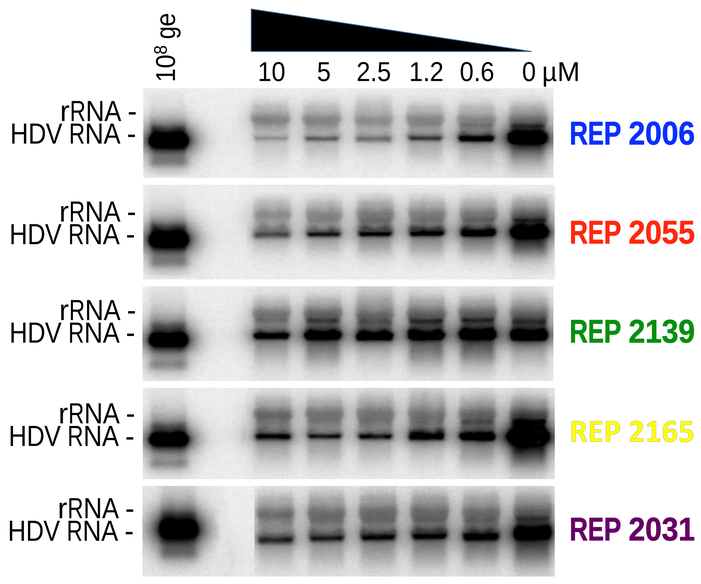
NAP	Sequence 5' - 3'	Length	Modifications			Chemistry
			PS	2'OMe	5'MeC	
REP 2006	N_{40} (degenerate)	40	a			Amphipathic
REP 2031	C_{40}	40	a			Amphipathic (inactivated at acid pH)
REP 2055	$(AC)_{20}$	40	a			Amphipathic
REP 2139	$(AC)_{20}$	40	a	a	a	Amphipathic
REP 2165	$(AC)_{20}$	40	a	a *	a	Amphipathic

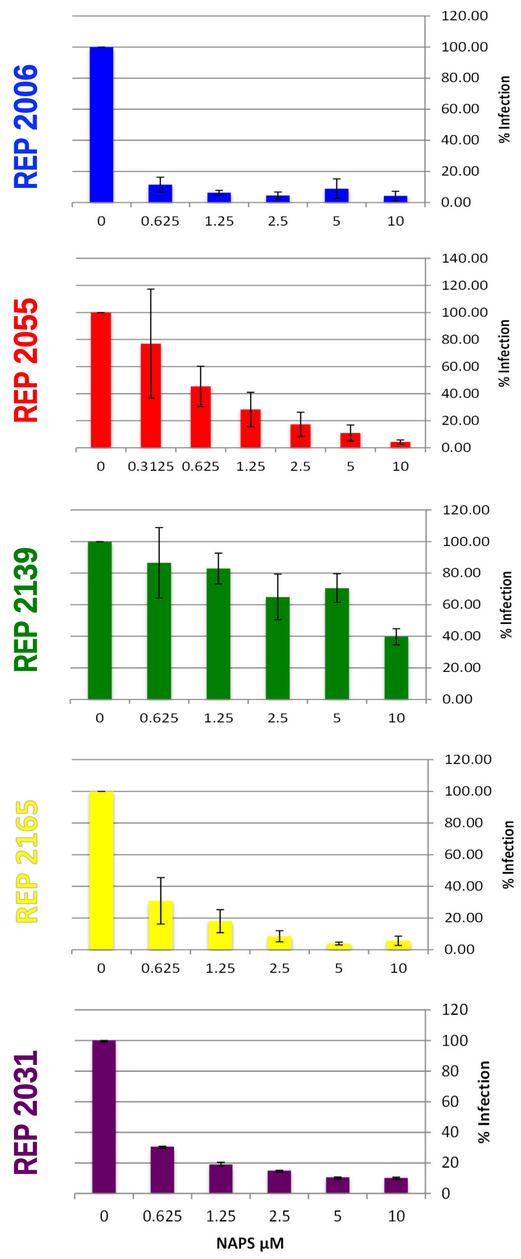
PS = phosphorothioation of phosphodiester linkage (increases amphipathicity)
2'OMe = O-linked methylation at 2' position in ribose (increased stability and reduced TLR reactivity)
5'MeC = methylation of 5' position in cytidine base (reduced TLR reactivity)
* Positions 11, 21 and 31 have 2'OH ribose

Active against HBV in clinical trials

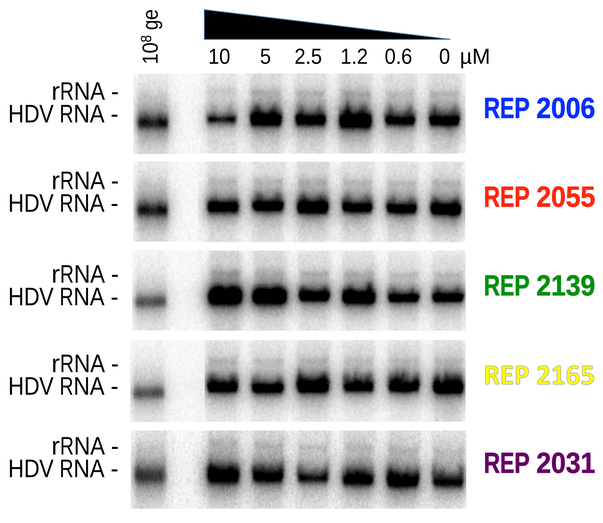
RESULTS

Entry / Huh7-NTCP

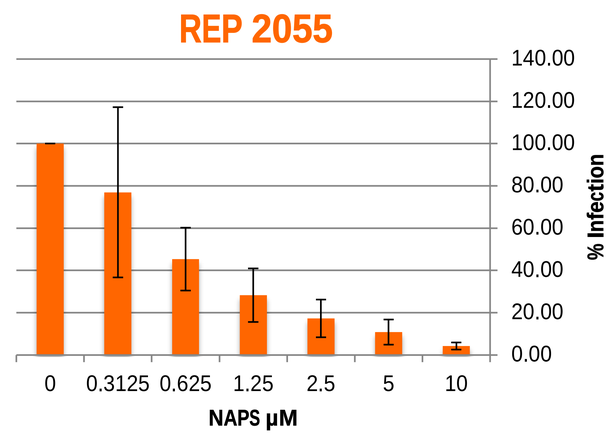




Post-Entry / Huh7-NTCP



Entry / Huh7-NTCP



Entry / HepaRG

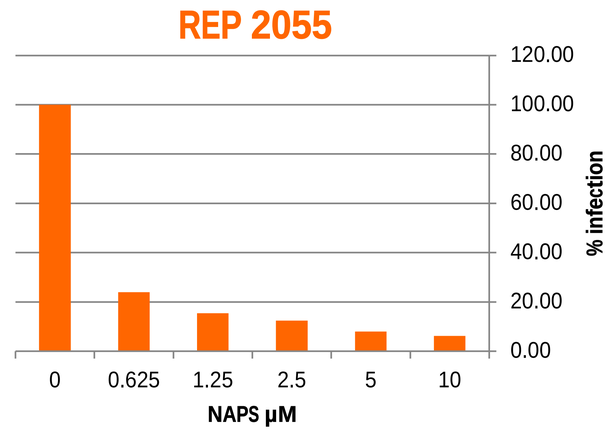


Figure 1. Entry inhibition of HDV into Huh-7-NTCP cells by various NAPs. Northern blotting of cellular HDV RNA in cells treated with NAPs coincoulation or post inoculation. Quantification from coinoculation experiments are shown. All NAPs demonstrate a dose dependent activity against HDV infection in NTCP-Huh-7 cells, with an IC50 ≤ 625 nM for the prototypic molecule (REP 2006). Data show that the antiviral effect is exerted at viral entry and not on HDV RNA replication. Treatment with NAPs showed no cytotoxicity at ≤ 10 µM concentrations.

Figure 2. Comparison of HDV entry inhibition into Huh-7-NTCP cells or HepaRG cells. REP 2055 demonstrate similar inhibitory potency in both Huh7-NTCP and differentiated HepaRG cells.

CONCLUSIONS & PERCEPECTIVE

- **NAPs display consistent antiviral activities against HDV entry *in vitro* (regardless of the cell type).**
- **Antiviral effect of NAPs is sequence-independent (consistent with DHBV studies).**

Ø **Next step will be to investigate a putative additional antiviral effect at a later stage of HDV lifecycle (suggested by clinical data)**

REFERENCES

1. Noordeen, F., et al, Antimicrob Agents Chemother. 57: 5291-8.
2. Noordeen, F., et al, Antimicrob Agents Chemother. 57: 5299-306.

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