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

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## BACKGROUND

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 in vitro, having both entry and post-entry activities (Noordeen et al., 2013). Additionally NAPs have been shown to be clinically active against HBV infection where they achieve rapid reductions in both serum HBsAg and HBV DNA.

## OBJECTIVES

HBV and hepatitis delta virus (HDV) are considered to have similar entry mechanisms, therefore the antiviral activity of various NAP compounds was assessed against HDV infection using two in vitro cellular infection models..

## MATERIALS & METHODS

The NAPs tested included REP 2006 (a prototypic NAP with a degenerate sequence), REP 2031, REP 2055, REP 2139 (clinically active against HBV) and REP 2165 (an additional clinical candidate NAP) – see table below. Differentiated HepaRG cells, or NTCP-expressing Huh-7 cells, were inoculated with HDV (MOI:100) in the presence of NAPs, and infection was monitored by measuring intracellular HDV RNA levels at day-9 postinoculation. Control experiments consisted in NAPS treatment applied postinoculation to specifically monitor for toxicity and inhibition of HDV RNA replication.

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

Treatment with NAPs resulted in the absence of cytotoxicity at ≤ 10 µM concentrations. All NAPs demonstrated a dose dependent activity against HDV infection in both HepaRG and NTCP-Huh-7 cells, with an IC50 ≤ 625 nM for the prototypic molecule. Data show that the antiviral effect was exerted at viral entry and not on HDV RNA replication. The antiviral effect of the prototypic, degenerate REP 2006 was comparable to that of NAPs with defined sequences.

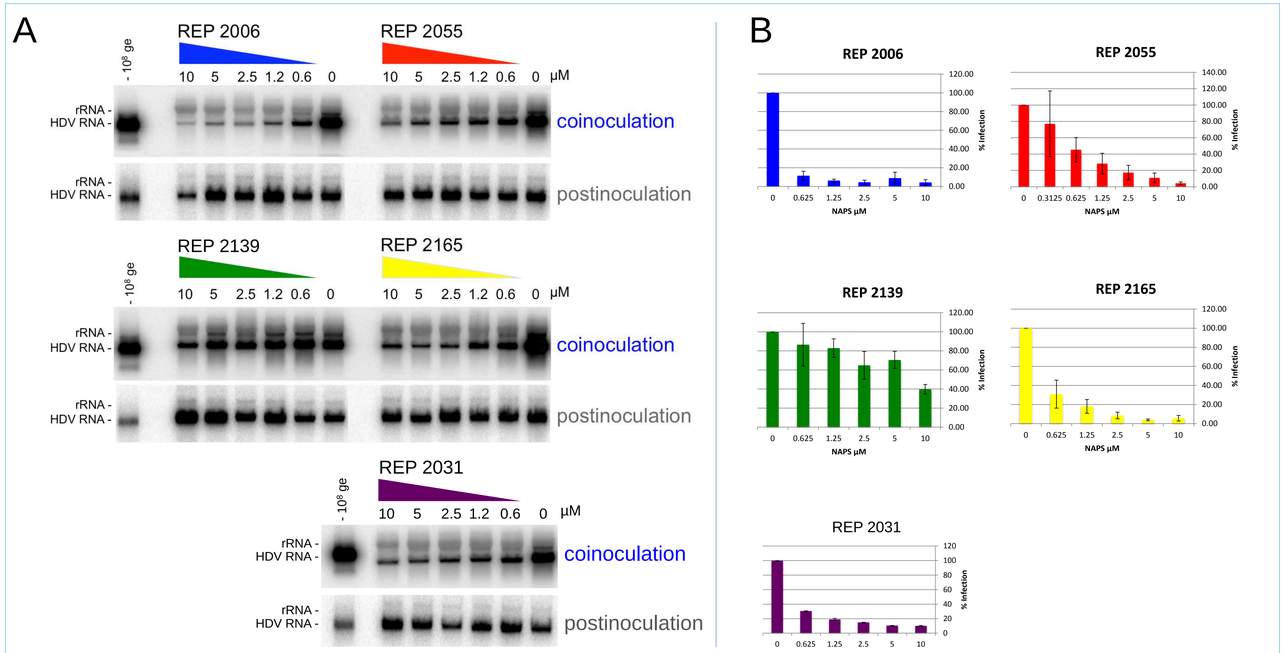


Figure 1. Entry inhibition of HDV into Huh-7-NTCP cells by various NAPs. A) Northern blotting of cellular HDV RNA in cells treated with NAPs coincoulation or post inoculation. B) Densitometry from coinoculation data from repeat experiments for REP 2006, REP 2055, REP 2139 and REP 2055 (n=3) and REP 2031 (n=2).

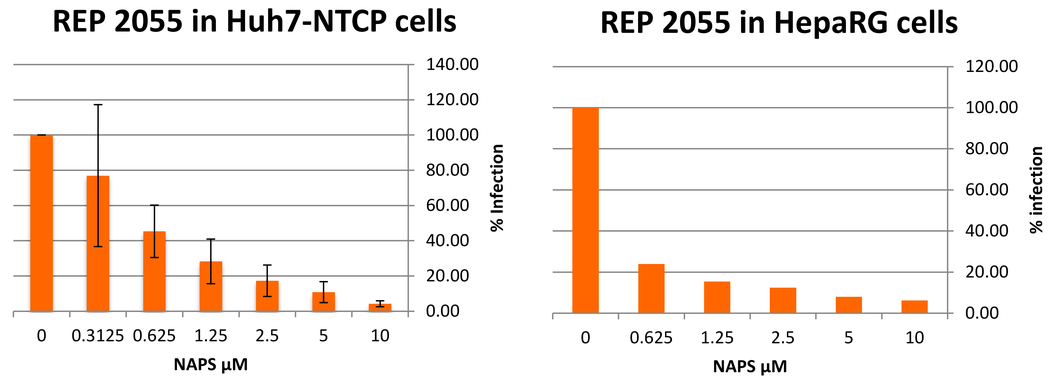


Figure 2. Comparison of entry inhibition of HDV into Huh-7-NTCP cells (n=3) or HepaRG cells (n=1) by REP 2055.

## SUMMARY

**Introduction:** 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 in vitro, having both entry and post-entry activities (Noordeen et al., 2013). Additionally NAPs have been shown to be clinically active against HBV infection in the where they achieve rapid reductions in both serum HBsAg and HBV DNA. Since HBV and hepatitis delta virus (HDV) are considered to have similar entry mechanisms, the antiviral activity of various NAP compounds was assessed against HDV infection using two in vitro cellular infection models.

**Methods:** The NAPs tested included REP 2006 (a prototypic NAP with a degenerate sequence), REP 2055, REP 2139 (clinically active against HBV) and REP 2165 (an additional clinical candidate NAP). Differentiated HepaRG cells, or NTCP- expressing Huh-7 cells, were inoculated with HDV in the presence of NAPs, and infection was monitored by measuring intracellular HDV RNA levels at day-9 postinoculation. Control experiments consisted in NAPS treatment applied postinoculation to specifically monitor for toxicity and inhibition of HDV RNA replication.

**Results:** Treatment with NAPs resulted in the absence of cytotoxicity at ≤ 10 µM concentrations. All NAPs demonstrated a dose dependent activity against HDV infection in both HepaRG and NTCP-Huh-7 cells, with an IC50 ≤ 625 nM for the prototypic molecule. Data show that the antiviral effect was exerted at viral entry and not on HDV RNA replication. The antiviral effect of the prototypic, degenerate REP 2006 was comparable to that of NAPs with defined sequences.

**Conclusions:** NAPs display an antiviral activity against HDV entry in vitro, which has the same sequence-independent antiviral effect as that observed for DHBV in vitro. These results suggest that NAPs are potent inhibitors of HDV infection.

## CONCLUSIONS

**NAPs display an antiviral activity against HDV entry in vitro, which has the same sequence-independent antiviral effect as that observed for DHBV in vitro. These results suggest that NAPs are potent inhibitors of HDV infection.**

## 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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