Activity of nucleic acid polymers against hepatitis D virus infection *in vitro*

P-144

Frauke Beilstein[1], Matthieu Blanchet[2], Andrew Vaillant[2], Camille Sureau[1]

1. Molecular Virology Laboratory (INTS), CNRS INSERM U1134, Paris, France.
2.Replicor Inc. Montréal, Canada, H4P 2R2

INTRODUCTION

The hepatitis D virus (HDV) infection is considered the most severe form of viral hepatitis in humans and one of the most difficult to cure for lack of efficient antiviral molecule. The virus life cycle is quite peculiar in that HDV is an obligate satellite of the Hepatitis B virus (HBV). The helper HBV confers propagation capacity to HDV by ensuring the supply of envelope proteins necessary to HDV virion assembly, cell egress, extra-cellular stability and infectivity. Hence, antivirals directed to the HBV helper functions are likely to be productive in suppressing HDV propagation. Furthermore, targeting viral entry as a therapeutic strategy should be effective against both HBV and HDV infections.

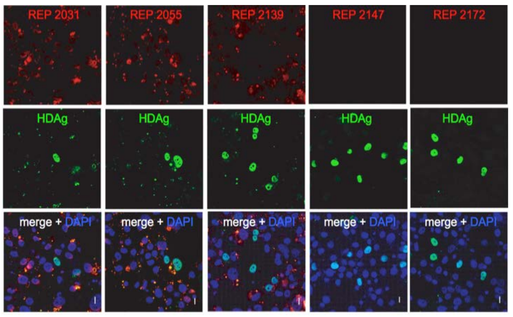
In this study, an in vitro HDV infection model was used to investigate the effects of nucleic acid polymers (NAPs), on HDV infection.

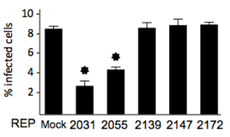
Nucleic acid polymers (NAPs)

NAP	sequence	length	PS	2'O Me	5-MeC
REP 2006	dN_{40}	40	+		
REP 2031	dC_{40}	40	+		
REP 2055	$(dAdC)_{20}$	40	+		
REP 2107	N_{40}	40	+	+	
REP 2138	C_{40}	40		+	
REP 2139	$(AC)_{20}$	40	+	+	+
REP 2165	$(AC)_{20}$	40	+	+*	+
REP 2147	$(AC)_{20}$	40		+	+
REP 2172	$(AC)_{20}$	40		+	

d: deoxynucleic acid (DNA),
N = degenerate sequence
PS: phosphorothioation of phosphodiester linkage
2'OMe: O-linked methylation at 2' position in ribose
5-MeC: methylation of 5' position in cytidine base
* Positions 11, 21 and 31 have 2'OH ribose

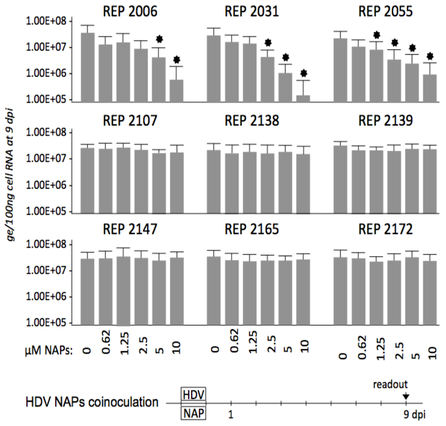
NAPs present during HDV inoculation are internalized and reduce % of infected cells





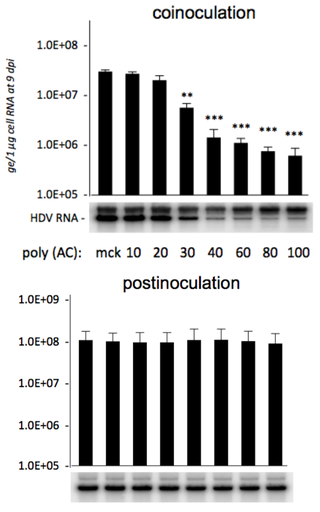
NTCP-expressing Huh-7 cells (Huh-106) were coinoculated with HDV and 10 µM of Cy3-tagged labelled NAPs (red). Infected cells were counted at 9 dpi using a human anti-HDAg antibody, and Alexa Fluor 488-conjugated anti-human IgGs (green). Scale bars, 20 µm. The ratio of infected cells were plotted as % of HDV infected cells.

NAPs inhibit HDV infection of human hepatocytes in culture



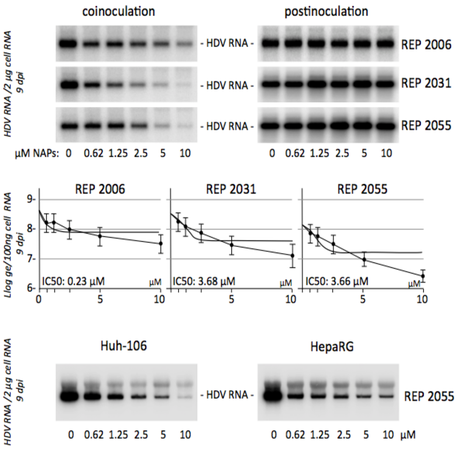
Huh-106 cells were exposed to HDV (500 ge/cell) in the absence (0) or presence of the indicated concentrations of NAPs. Intracellular HDV RNA at 9 days postinoculation (dpi) was quantified by real-time qRT-PCR for measurement of infection. Values in the histograms are shown as means ± SD, expressed as ge per 100 ng of cellular RNA, in three independent experiments.

NAPs inhibitory acitivity is size-dependent



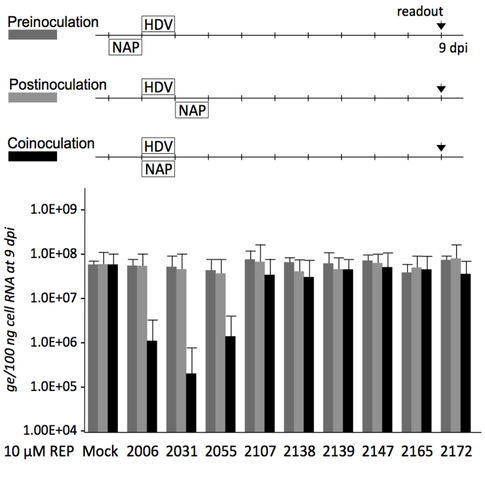
Cells were inoculated with HDV (500 ge/cell), in the absence or the presence of 10 µM of 100- to 10-mer versions of REP 2055 under two conditions i) a coinoculation treatment ii) postinoculation treatment. At 9 days postinoculation, total cellular RNA was isolated and analyzed for the presence of HDV RNA by northern blot. The values in the histograms are shown as means ± SD, expressed as genome equivalents (ge) per µg of cellular RNA, obtained in three independent experiments.

NAPs are active against HDV at submicromolar concentrations



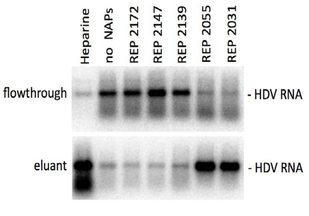
Huh-106 or HepaRG cells were inoculated with HDV (500 ge/cell), in the absence (0) or the presence of NAPs in : i) a coinoculation treatment, and ii) postinoculation treatment in which NAPs were added to cells for 24 h, after removal of the inoculum. At 9 dpi, intracellular HDV RNA was measured by northern blot. The IC50 was calculated using GraphPad Prism.

NAPs block HDV entry but do not interfere with HDV RNA replication post entry



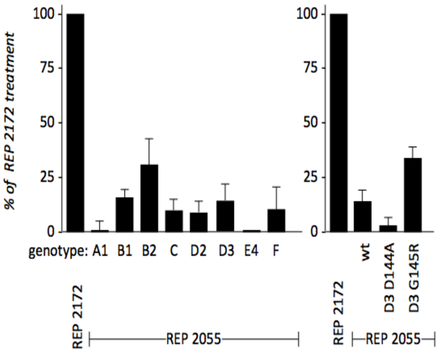
Huh-106 cells were inoculated with HDV (500 ge/cell), in the absence or the presence of 10 µM NAPs, under 3 conditions i) preinoculation: cells exposed to NAPs for 24 h prior to HDV, ii) postinoculation: treatment of cells with NAPs for 24 h, after removal of HDV. iii) coinoculation, in which cells were exposed to HDV and NAPs for 24 h. Intracellular HDV RNA at 9 dpi was quantified by real-time qRT-PCR . Values in the histograms are means ± SD, expressed as ge/100 ng of total cellular RNA, in three independent experiments.

HDV virions bind to immobilized NAPs



Biotinylated NAPs were bound to streptavidin-sepharose in a 400 µl volume column. After extensive washes in 150 mM NaCl solution, HDV-containing supernatants were loaded on the column. Flowthrough was recovered; columns were washed 5 x with 150 mM NaCl, and bound particles were eluted with 1 M NaCl solution. HDV RNA was measured by northern blot analysis in flowthrough and eluate fractions.

NAPs are active against HDV bearing HBV immune escape substitutions (D144A and G145R) and pangenomic with regard to HBV envelope proteins.



Huh-106 cells were exposed to HDV virions (500 ge/cell) containing the envelope proteins of the indicated HBV genotypes, or particles genotype D envelope proteins bearing the substitutions D144A, or G145R in the presence of 10 µM of REP 2055 or REP 2172. Intracellular HDV RNA extracted at 9 days postinoculation (dpi) was quantified by northern blot analysis for measurement of infection. The values in the histograms are shown as means percentage ± SD of infections in the presence of REP 2172 control, obtained in three independent experiments.

CONCLUSIONS

- The DNA-based NAPs, REP 2006, REP 2031 and REP 2055 inhibit HDV infection *in vitro* at submicromolar concentrations. The RNA-based NAPs REP 2139 and REP 2165 are inactive in inhibiting HDV infection.
- NAPs are active at viral entry, but inactive post entry on HDV RNA replication in the model systems employed.
- Inhibition is independent of the nucleotide sequence of NAPs, but dependent on both size and amphipathicity of the polymer.
- NAP antiviral activity is effective against HDV virions bearing the main HBV immune escape substitutions (D144A and G145R) and was pangenomic with regard to HBV envelope proteins.
- Similar to immobilized heparin, immobilized NAPs bind HDV particles suggesting that entry inhibition is due, at least in part, to preventing the initial attachment of the virus to cell surface glycosaminoglycans.
- The results document NAPs as a novel class of antiviral compounds that can prevent HDV propagation.

REFERENCES

Noordeen et al., 2013a Antimicrob. Agents Chemother. 57: 5299-5306;
Noordeen et al., 2013b Antimicrob. Agents Chemother. 57: 5291-5298;
Noordeen et al., 2015 PLOS One 10;
Al-Mahtab et al., 2016 PLOS One 11;
Vaillant, 2016 Antiviral Res. 133: 32-40

CONTACT

Camille Sureau: sureau@ints.fr
Andrew Vaillant: availlant@replicor.com