Post-entry antiviral effects of nucleic acid polymers against HBV infection *in vitro*

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INTRODUCTION

- Nucleic acid polymers (NAPs) are phosphorothioated oligonucleotides that have demonstrated a great potential to inhibit HBV and HDV infection.
- NAPs are potent suppressors of serum HBsAg *in vivo* in the duck model and in proof of concept clinical trials..
- Certain NAPs exert antiviral properties at the entry step of HBV in vitro. However, REP 2139, which is clinically active, has no entry activity.
- The post entry effect appears to be essential for targeting HBsAg secretion and for clinical effect.
- NAPs, as for other phosphorothioated oligonucleotides (i.e. antisense), do not exert significant activity within the cell when provided without a delivery system. This has made the demonstration of a post-entry effect of NAPs in previous *in vitro* studies difficult.

AIM

To develop an *in vitro* model that replicates the post entry antiviral effect of NAPs in HBV infection *in vivo* and in the clinic to further characterize the molecular targets involved in the inhibition of HBsAg secretion by NAPs.

METHODS

- HepG2.2.15 cells stably expressing HBV were used in this study.
- Cell culture, treatment and harvesting were conducted as described in each result panel.
- Electroporation (Ep) parameters (apparatus name) were as follows: 4 pulses of 99 uS at 820V with a 1.1 s interval.
- ELISA kits used in this study are as follows: HBsAg, Murex version 3 (Diasorin); hAlbumin, Abcam ab197887.
- PCSK9 mRNA was quantified by reverse transcription using iScript select cDNA Synthesis kit (Biorad) and qPCR using Ssofast Evagreen supermix (Biorad) with the following primers : ggatacctcaccaagatcct, R-caacttcaaggccagctc.
- HBV virions from the supernatant were immunoprecipitated using an anti-preS1 antibody (Santa Cruz,sc-57761) and protein A/G agarose beads (Santa cruz, sc-2003). Viral DNA was extracted using QIAamp DNA mini kit (Qiagen) and quantified by Taqman qPCR using Ssoadvanced probe universal supermix (Biorad). Primers used were as follows: F-acgtcctttgtttacgtcccg, R-ccaactcctcccagtctttaaac Probe sequence was as follows: FAM-tcaacgaccgaccttga-dabcyl-MBG.
- Cellular HBV RNA was monitored by reverse transcription and Taqman qPCR as described above.
- Results were normalized to the concentration of total cellular protein in cell lysates, as measured by BCA.
- Cell viability was measured using the CellTiter 96 Aqueous One Solution Cell proliferation assay (Promega) and is expressed relative to the normal saline electroporation.

NAPs used in the study

Name	NAPs Sequence (5' – 3')	Chemical Modifications		
		PS	2'OMe	5-MeC
REP 2055	ACACACACACACACACACACACACACACACACACACAC (40 mer)	+		
REP 2139	ACACACACACACACACACACACACACACACACACACAC (40 mer)	+	+	+
REP 2147	ACACACACACACACACACACACACACACACACACACAC (40 mer)		+	+

PS = phosphorothioate, 2'Ome = 2' O methylation of ribose, 5-MeC = 5' methylation of cytosine base

RESULTS

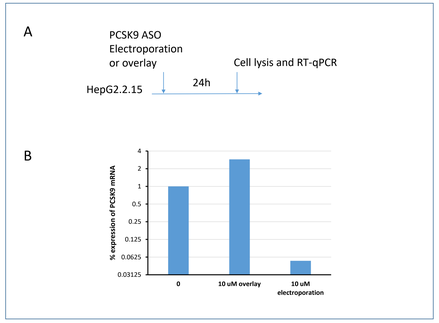

Fig. 1. A delivery system is required for antisense activity in HepG2.2.15 cells. (A) treatment paradigm. (B) Effect of electroporation on antisense effect.

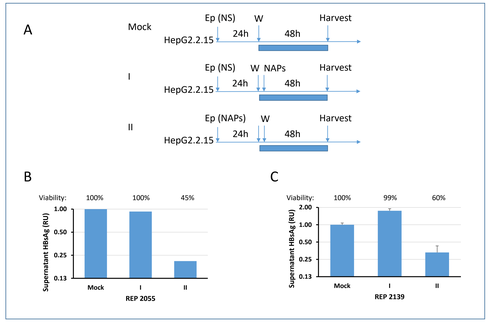

Fig. 2. Electroporation is required for NAP antiviral activity in HepG2.2.15 cells. A) treatment paradigm. HBsAg reduction in the supernatant with 25 uM REP 2055 (B) or 150 uM REP 2139 (C). Ep, electroporation; W, wash; NS, normal saline.

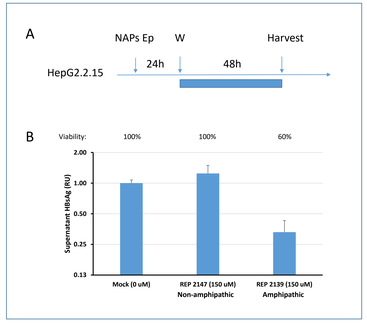

Fig. 3. Phosphorothioation of NAPs (amphipathicity) is mandatory for antiviral activity. Ep, electroporation; W, wash.

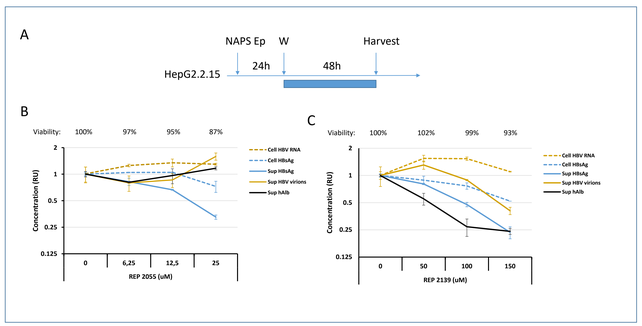

Fig. 4. Effect of HepG2.2.15 electroporation with NAPs on HBV lifecycle, and on albumin secretion. (A) treatment paradigm. (B) and (C) results with REP 2055 and REP 2139, respectively. Ep, electroporation; W, wash.

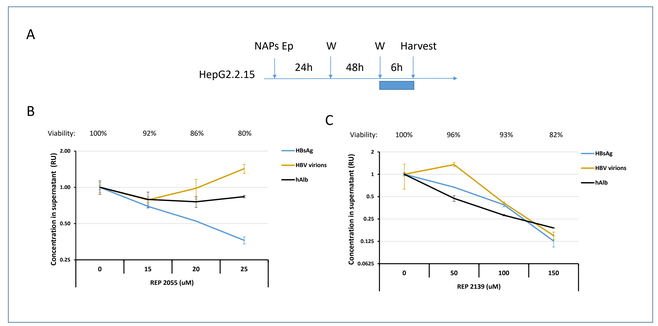

Fig. 5. Effect of HepG2.2.15 electroporation with NAPs on HBV lifecycle, and on albumin secretion. (A) treatment paradigm. (B) and (C) results with REP 2055 and REP 2139, respectively. Ep, electroporation; W, wash.

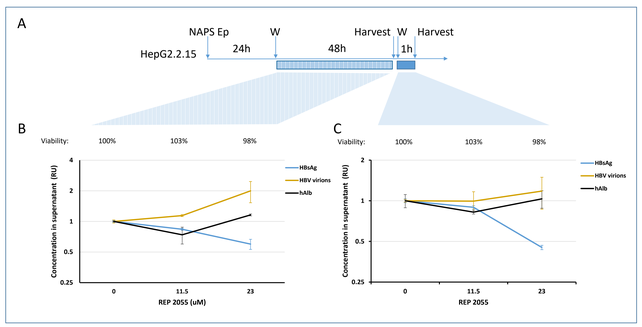

Fig. 6. Effect of HepG2.2.15 electroporation with REP 2055 on HBV lifecycle and on albumin secretion. (A) treatment paradigm. (B) and (C) results with accumulation of 48 h and 1h, respectively. Ep, electroporation; W, wash.

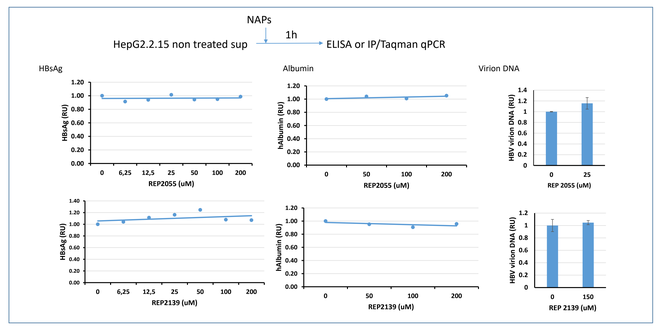

Fig. 7. Spiking experiments. NAPs do not interfere with ELISA quantification of HBsAg and Albumin, or with IP-extraction-Taqman quantification of virion-derived DNA

CONCLUSIONS

- Electroporation of NAPs in HepG2.2.15 cells can reproduce the post-entry antiviral effects of NAPs observed in vivo and in patients.
- REP 2055 selectively inhibits HBsAg secretion without affecting intracellular HBsAg levels, virion maturation and egress, or albumin secretion.
- These effects suggest that NAPs act by a post-translational mechanism that selectively interferes with assembly and or egress of SVP.
- In this model, REP 2139 displays a broader activity, leading to decreased concentrations of HBsAg, HBV virions and albumin in the supernatant without major alteration of cell viability.
- The REP 2139 effects observed in vitro in this model differs from its observed *in vivo* and clinical activity, where REP 2139 exerts a selective effect on SVP secretion similar to REP 2055.

ACKNOWLEDGEMENTS

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DISCLOSURES

MB and AV are employees of Replicor Inc. AV is a shareholder in Replicor Inc. PL has nothing to disclose.

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