Improved *in vitro* assay for investigating the post-entry activity of nucleic acid polymers *in vitro* against HBV infection

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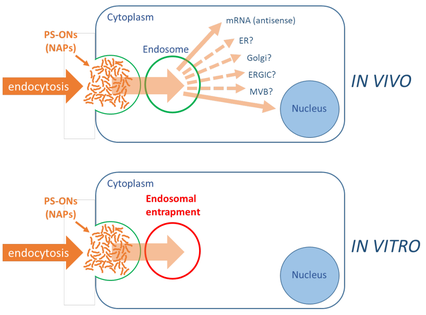
BACKGROUND & AIMS

- Nucleic acid polymers (NAPs) are phosphorothioated oligonucleotides (PS-ONs) that inhibit HBV[1,2] and HDV[3] infection.
- NAPs act via a post-entry mechanism to block the release of HBV subviral particles[4,5], effectively clearing HBsAg from the circulation[1,2], an effect which may also participate in the antiviral effects of NAPs in HDV.
- Similar to other PS-ONs, the intracellular trafficking of NAPs *in vivo* is altered *in vitro*[6-8], preventing the observation of any post-entry effects in the absence of delivery system.
- An electroporation-based protocol to facilitate NAP transit *in vitro* resulted in a post-entry inhibition of HBsAg secretion in the µM range[5].
- **An electroporation-free method for restoration of NAP transit *in vitro* is presented which results in a post-entry inhibition of HBsAg secretion in the nM range.**

METHODS

- Electroporation (Ep) parameters of HepG2.2.15 cells were as follows: 4 pulses of 99 uS at 820V with a 1.1 s interval.
- Endosomal release was achieved by adding 10uM UNC 7938[7] for 2 h after overnight exposure of cells to NAPs.
- ELISA kits used in this study are as follows: HBsAg, Murex version 3 (Diasorin); HBeAg,ETI-EBK plus, N0140, DIASORIN; hAlbumin, Abcam ab179887.
- BIP/GRP-78 and SKI-1/S1P mRNA were quantified by reverse transcription using iScript select cDNA Synthesis kit (Biorad) and qPCR using Ssofast Evagreen supermix (Biorad).
- 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).
- Cellular HBV RNA was monitored by reverse transcription and Taqman qPCR following total RNA extraction.
- Results were normalized to the concentration of total cellular protein in cell lysates, as measured by BCA or to cell viability, as measured using the CellTiter 96 Aqueous One Solution Cell proliferation assay (Promega).

The NAP transit problem

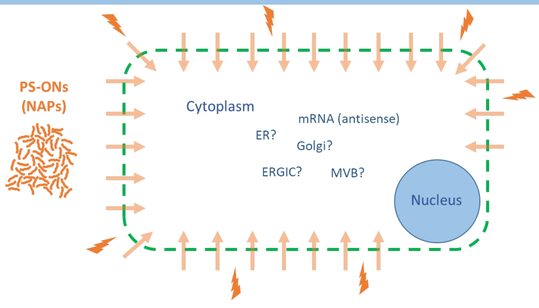


***In vivo* trafficking of PS-ONs (NAPs) is attenuated *in vitro*[6-8].**

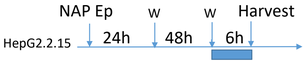
RESULTS

Electroporation

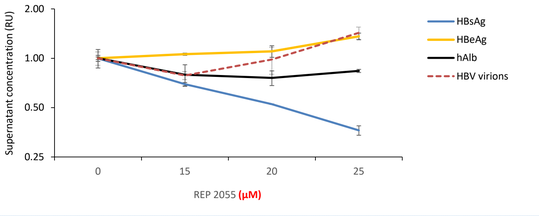
Restoration of NAP transit



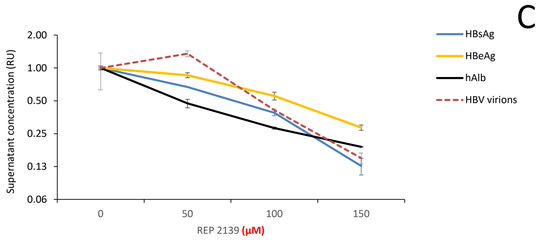
Cell handling



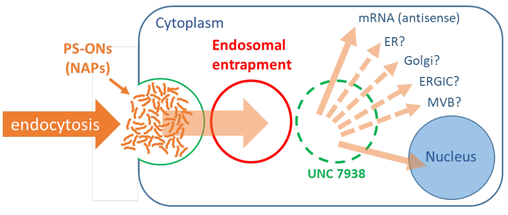
REP 2055

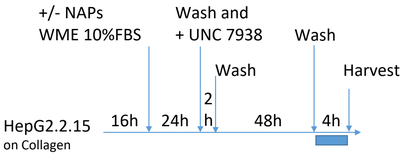


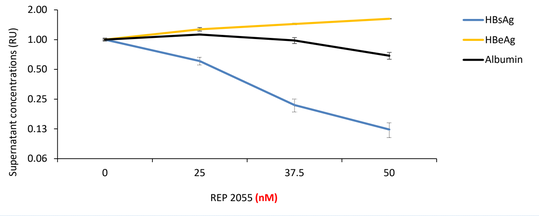
REP 2139

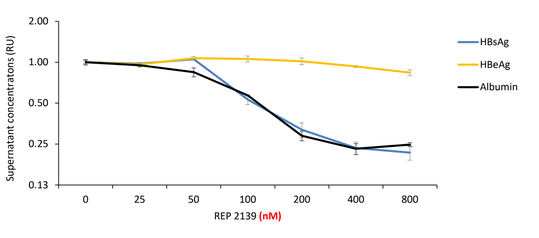


Endosomal release (UNC 7938[8])

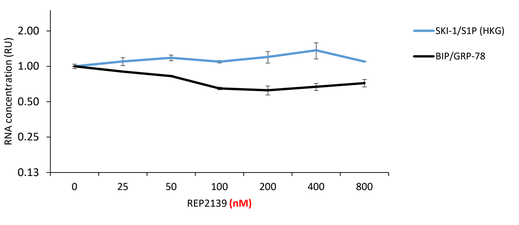








Additional investigations for panel D



NAPs used in this study

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

CONCLUSIONS & PERSPECTIVE

- **Endosomal release of NAPs with UNC 7938 reproduces the post-entry antiviral effects of NAPs observed *in vivo* and in patients at concentrations considerably lower than with electroporation (Panel B vs A and D vs C).**
- **The IC50 for post-entry activity of REP 2055 (~37.5nM) is substantially stronger than for inhibiting viral entry (~1uM)[9], suggesting that entry inhibition by this NAPs plays a minor role in antiviral effects observed *in vivo*.**
- **NAPs act by a post-translational mechanism that selectively interferes with assembly and or egress of SVP.**
- **REP 2139 displays a broader activity, with decreased concentrations of HBsAg and albumin in the supernatant without major alteration of cell viability however HBeAg secretion is not significantly altered (panel D).**
- **The reduced concentration of albumin in the supernatant does not appear to result from ER stress (Panel E) and is inconsistent with the lack of effect of REP 2139 on serum albumin *in vivo* and in patients during chronic therapy.**

REFERENCES

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CONTACT INFORMATION

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