Antiviral effects of nucleic acid polymers on hepatitis B virus infection *in vitro*

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Background & Aims

Nucleic acid polymers (NAPs) inhibit duck hepatitis B virus (DHBV) infection *in vivo* (Noordeen et al., 2013) and HBV infection in recent proof of concept clinical trials. While the antiviral effect of NAPs has been consistently shown to include the elimination of HBsAg (or DHBsAg) from the blood, the mechanism behind this effect still remains to be clearly elucidated. In this study, the previously established antiviral activity of various NAP analogs was compared with their antiviral effects in HBV infected HepaRG and primary human hepatocytes (PHH). Importantly, we also show their inhibition of subviral particle (SVP) assembly in sHBsAg expressing BHK-21 cells.

Materials & Methods

HepaRG cells (Hantz et al., 2009) and PHH (Gripon et al., 1988) were treated with NAPs every two days starting at the time of infection with HBV. The Elecsys HBsAg ELISA assay was used to quantitatively measure secreted HBsAg. HBeAg and PreS1 containing particles were also assessed by ELISA. Intracellular HBV RNA was measured by RT-PCR. *In vitro* toxicity was assessed by measuring the neutral red uptake (Repetto et al., 2008). Expression of S-HBsAg in BHK-21 cells was driven by electroporation of a SFV-derived template RNA. NAPs were added during the electroporation process. Expression and localization of S-HBsAg was monitored by immunofluorescence microscopy (Patient et al., 2007).

NAPs used in the present study

Name	Sequence 5' - 3'	Length	Modifications			Chemistry
			PS	2'OMe	5'MeC	
REP 2055	$(AC)_{20}$	40	+			amphipathic (no CpG)
REP 2006	$(N)_{40}$ (degenerate)	40	+			amphiapthic (contains CpG)
REP 2031	$(C)_{40}$	40	+			amphipathic (no CpG, neutralized at acidic pH due to tetramerization)
REP 2139	$(AC)_{20}$	40	+	+	+	amphipathic (no CpG)
REP 2165	$(AC)_{20}$	40	+	+*	+	amphipathic (no CpG - REP 2139 variant designed to degrade more rapidly)
REP 2138	$(C)_{40}$	40		+		polyanionic (no CpG, inactive control)

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

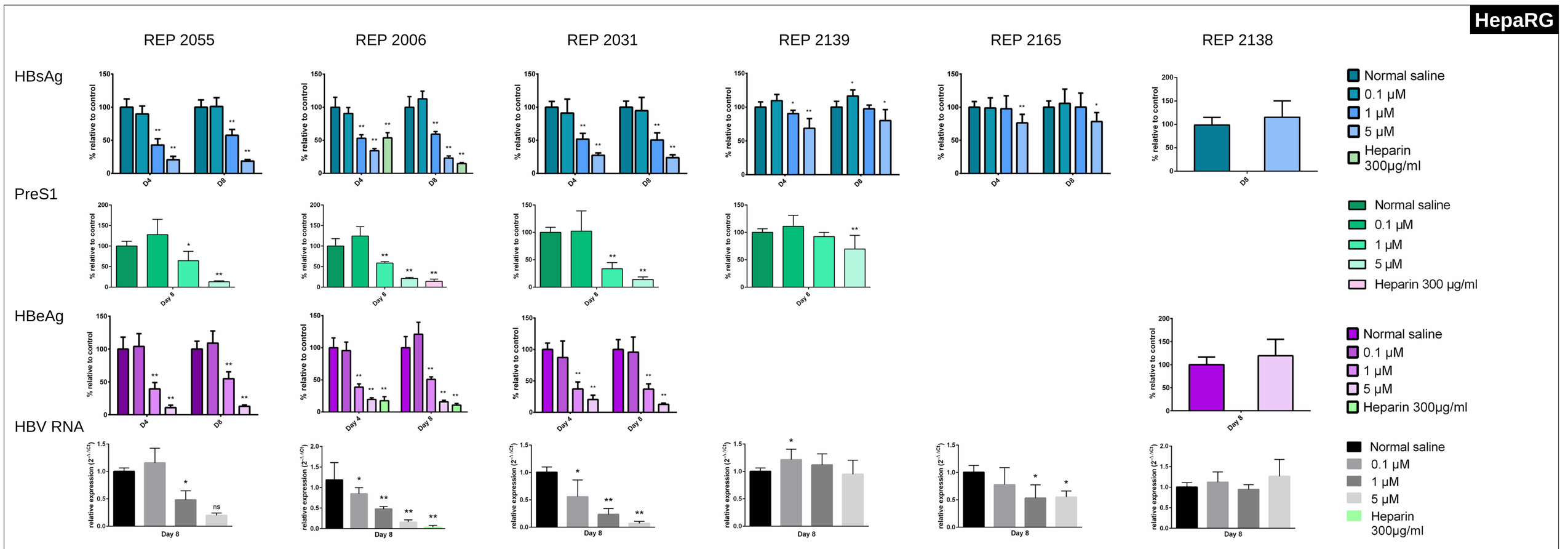

Figure 1 : Antiviral activity of several NAPs compounds on HBV entry. Differentiated HepaRG cells were infected and treated with NAPs as described above. At the indicated time, supernatant was tested for the presence of extracellular HBsAg, PreS1 containing particles, HBeAg. Cellular total HBV RNA was also quantified. NAPs significantly reduced extracellular HBsAg, HBeAg, and PreS1 containing particles as well as total HBV RNA in a dose dependent manner. REP 2055, REP 2031 and REP 2006 induced a 80% decreased of all viral parameters at 5 µM and REP 2139 and REP 2165 induced a 20% decreased. (Normal Saline solution, no NAP).

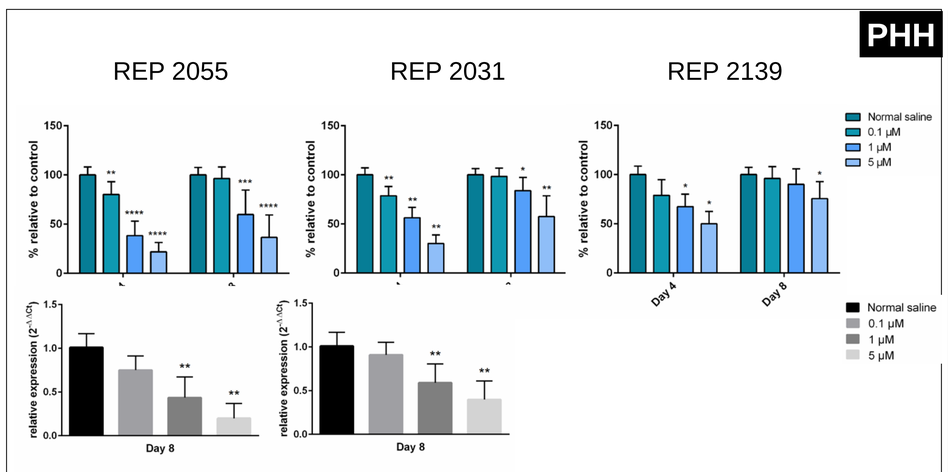

Figure 2 : Antiviral activity of NAPs on HBV entry on primary human hepatocytes. REP 2055 and REP 2031 exert a strong decrease of HBsAg and total HBV RNA. REP 2139 showed a mild antiviral activity. (Normal Saline solution, no NAP).

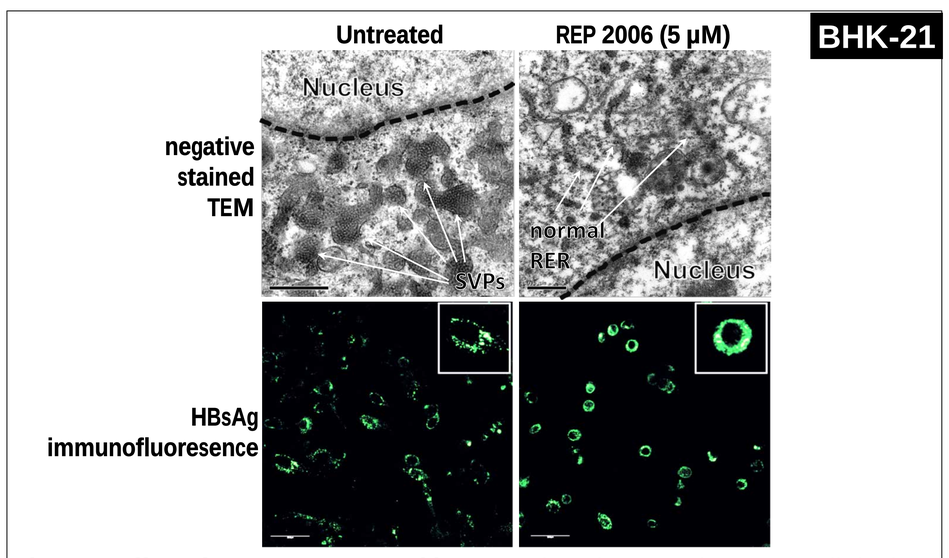

Figure 3 : Effect of NAPs on SVP assembly. Expression of S-HBsAg in BHK-21 cells in the absence of NAP results in the formation of SVPs in perinuclear vesicles (upper left). HBsAg expression in the cells (bottom left) exhibits the expected punctate pattern. NAPs electroporation in BHK-21 cells block the assembly of SVPs (upper right) and result in the retention of HBsAg in the perinuclear space (bottom right).

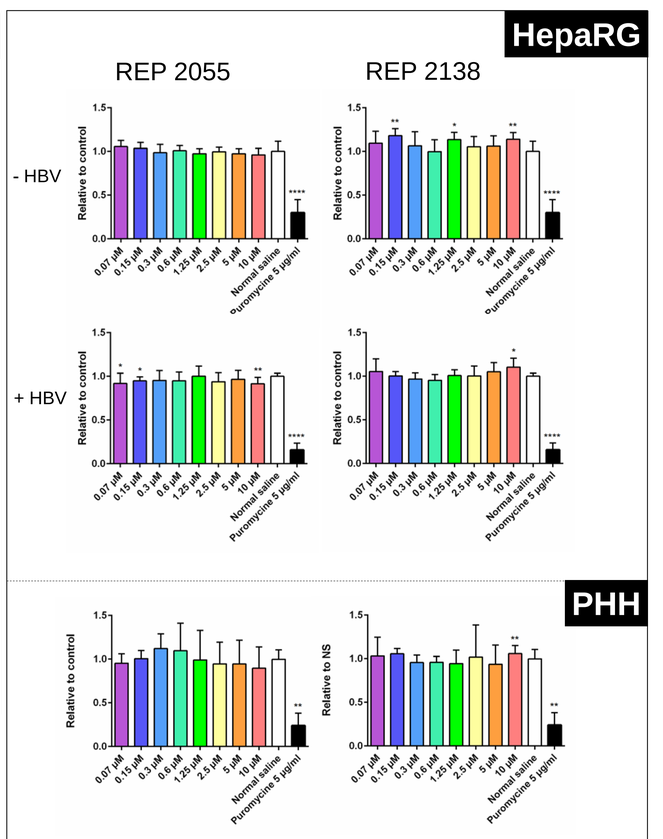

Figure 5 : **Toxicity of NAP compounds** was measured using the neutral red uptake method. Toxicity was assessed in differentiated HepaRG cells infected (+ HBV) or not (- HBV) and in PHH (B). No evidence of toxicity was observed with NAPs at concentrations up to 10 µM.

Conclusions

A strong antiviral activity of NAPs against HBV entry in HepaRG cells and primary human hepatocytes and in restricting HBsAg to the perinuclear space in BHK-21 cells was observed. The phosphorothioation (amphipaticity) dependent antiviral activity of NAPs was well correlated with the previously established antiviral activity in ducks and in human patients with chronic HBV infection. These results suggest that the clearance of HBsAg (or DHBsAg) in the blood with NAP treatment may be linked to the inhibition of SVP assembly and re-infection of hepatocytes. These antiviral activities both on virus entry and within the cells promise a strong potential of NAPs alone or in combination with already existing antiviral treatments.

References

Noordeen, F., A. Vaillant, et al. "Nucleic acid polymers prevent the establishment of duck hepatitis B virus infection in vivo." Antimicrob Agents Chemother **57**(11): 5299-306.
Hantz, O., R. Parent, et al. (2009). "Persistence of the hepatitis B virus covalently closed circular DNA in HepaRG human hepatocyte-like cells." J Gen Virol **90**(Pt 1): 127-35.
Gripon, P., C. Diot, et al. (1988). "Hepatitis B virus infection of adult human hepatocytes cultured in the presence of dimethyl sulfoxide." J Virol **62**(11): 4136-43.
Patient, R., C. Hourioux, et al. (2007). "Hepatitis B virus subviral envelope particle morphogenesis and intracellular trafficking." J Virol **81**(8): 3842-51.
Repetto, G., A. del Peso, et al. (2008). "Neutral red uptake assay for the estimation of cell viability/cytotoxicity." Nat Protoc **3**(7): 1125-31.

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