Antiviral effects of nucleic acid polymers on hepatitis B virus infection

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BACKGROUND

Hepatitis B virus (HBV) infection remains a major public health problem worldwide. None of the current therapies are able to cure HBV infection. Nucleic acid polymers (NAPs) have been shown to inhibit duck HBV infection *in vitro* and *in vivo* (Noordeen et al., 2013). NAPs are amphipathic oligonucleotides constructed from phosphorothioation of a nonbridging oxygen atom in the phosphodiester linkage. This amphipathic property allows interactions of NAPs with structurally conserved amphipathic alpha-helical protein domains such as type 1 viral fusion glycoproteins and display demonstrated antiviral activity against several viruses. Due to their phosphorothioated structure, NAPs are chemically analogous to sulfated polyglycans as heparin which has been shown to block entry of hepatitis B virus.

OBJECTIVES

In this study we investigated the *in vitro* antiviral activity of NAPs in HBV infected HepaRG cells and primary human hepatocytes.

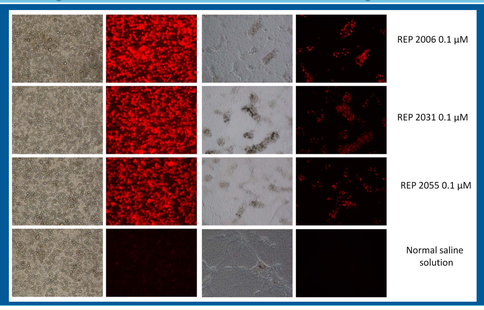
MATERIALS & METHODS

NAPs uptake was assessed using Cy3 labeled NAPs. In order to evaluate potent effects of NAPs on HBV entry as well as post-entry infection, HBV infected differentiated HepaRG cells (Hantz et al., 2009) and primary human hepatocytes (PHH) were treated with NAPs every two days starting at the time of infection or two days post-infection. The Elecsys HBsAg II quant automated system was used to quantitatively measure the secreted HBsAg. PreS1 containing particles and HBeAg were also assessed by ELISA. NAPs used were as follows:

NAP	Sequence 5' - 3'	Length	Modifications			Chemistry
			PS	2'OMe	5'MeC	
REP 2138	C_{40}	40		✔		Polyanionic (inactive control)
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

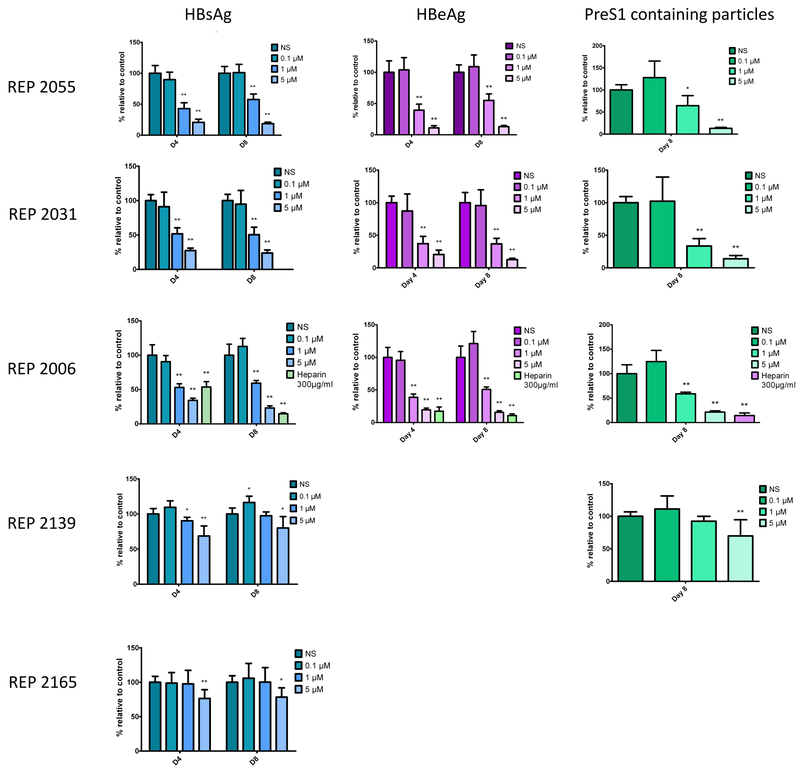
PS = phosphorothioation of phosphodiester linkage (increases amphipathicity)
2'OMe = O-linked methylation at 2' position in ribose (increased stability and reduced TLR reactivity)
5'MeC = methylation of 5' position in cytidine base (reduced TLR reactivity)
* Positions 11, 21 and 31 have 2'OH ribose
Active against HBV in clinical trials

NAP uptake in PHH and HepaRG cells

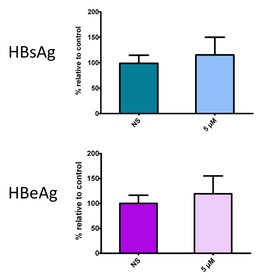


Uptake of several NAPs was assessed in primary human hepatocytes (left) and differentiated HepaRG cells (right) using Cy3 labeled NAPs. Bright field and Cy3 staining are shown. (Normal saline solution : compound solvant as a non treated condition).

Antiviral effect of NAPs added during HBV entry in HepaRG cells



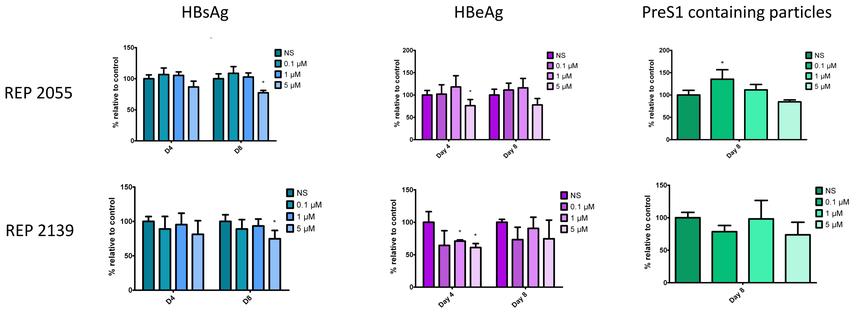
REP 2138 inactive control



The polyanionic (non-amphipatic) inactive control NAP REP 2138 was used to demonstrate the specific antiviral activity of amphipatic NAPs. REP 2138 did not show any antiviral activity assessed by measuring extracellular HBsAg and HBeAg. Data expressed as means ± standard deviation were obtained from three independent experiments.

Antiviral activity of several NAPs compounds on HBV entry was assessed in differentiated HepaRG cells by measuring extracellular HBsAg, PreS1 containing particles and HBeAg. All NAPs showed a dose dependent antiviral activity with REP 2055, REP 2031 and REP 2006 inducing a 80% decreased of all viral parameters at 5 µM. REP 2139 and REP 2165 induced a 20% decreased of viral parameters. Data expressed as means ± standard deviation were obtained from three independent experiments. P values were calculated by non parametric Mann-Whitney test between two groups (NS vs. compound concentration tested). *, $p < 0.05$; **, $p < 0.01$.

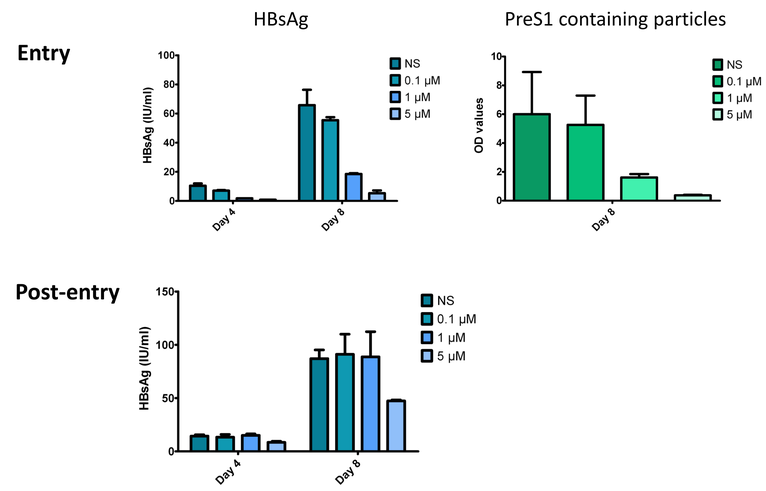
Antiviral effect of NAPs added post HBV entry in HepaRG cells



Post HBV entry antiviral activity of several NAPs compounds was assessed by measuring extracellular HBsAg, PreS1 containing particles and HBeAg. REP 2055 and REP 2139 showed a post-entry antiviral activity with a 20% decreased in viral parameters assessed. Data expressed as means ± standard deviation were obtained from two independent experiments. P values were calculated by non parametric Mann-Whitney test between two groups (NS vs. compound concentration tested).
*, $p < 0.05$; **, $p < 0.01$.

Antiviral activity of NAPs in PHH

REP 2055



Antiviral activity of REP 2055 was assessed in primary human hepatocytes by measuring extracellular HBsAg and PreS1 containing particles. REP 2055 induced more than 90% of HBsAg and PreS1 containing particles decrease when added at the time of infection and a 50% decrease of HBsAg release when added 2 days post-infection. Data are expressed as means ± standard deviation obtained from duplicates of one experiment.

CONCLUSIONS

In this study, we showed a strong antiviral activity of nucleic acid polymers against HBV infection in HepaRG cells and primary human hepatocytes. Our results suggest that :

- NAPs enter specifically into hepatocytes (rather than biliary cells)
- NAPs are able to block entry of HBV in a sequence independent manner
- NAPs affect the replication cycle following entry of the virus

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.

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