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 alphahelical 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 Electrop 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:

<table>
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<tr>
<th>NAP</th>
<th>Sequence 5’ - 3’ Length</th>
<th>Modifications</th>
<th>Chemistry</th>
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</thead>
<tbody>
<tr>
<td>REP 2138</td>
<td>5’ C40 40</td>
<td></td>
<td>Amphilic</td>
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<tr>
<td>REP 2006</td>
<td>5’ C40 40</td>
<td></td>
<td>Amphipathic</td>
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<tr>
<td>REP 2005</td>
<td>5’ C40 40</td>
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<td>Amphipathic</td>
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<tr>
<td>REP 2139</td>
<td>5’ C40 40</td>
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<td>Amphipathic</td>
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<td>REP 2165</td>
<td>5’ C40 40</td>
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<td>Amphipathic</td>
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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% decrease of all viral parameters at 5 µM. REP 2139 and REP 2165 induced a 20% decrease of viral parameters. Data expressed as means ± standard deviation were obtained from three independent experiments.

RESULTS

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% decrease 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.

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.

REFERENCES

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