Therapeutic effect against hepatitis B of various nucleic acid polymers in the chronic DHBV infection model

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EASL The Home of Hepatology
50th THE INTERNATIONAL LIVER CONGRESS 2015
APRIL 22-26, VIENNA, AUSTRIA

BACKGROUND

Nucleic acid polymers (NAPs) block the release of HBsAg from infected hepatocytes appearing therefore of particular interest for chronic hepatitis B therapy. Two current NAPs compounds (REP 2055 and REP 2139), effectively clear the bloods of HBsAg in human subjects with chronic HBV infection and when used in combination with immunotherapy have been able to achieve higher SVR rates in patients than when immunotherapy is used alone.

OBJECTIVES

The goal of this preclinical study was to examine the effect of various nucleic acid modifications on the tolerability, liver accumulation and antiviral effect of NAPs in vivo, in chronic DHBV infection model.

MATERIALS & METHODS

Three-day-old Pekin ducklings were infected with $2x10^{11}$ VGE/ml of DHBV from infectious duck serum. NAP treatment was started in 14 days-old animals and consisted of dosing via intraperitoneal injection with 10mg/kg of NAPs (formulated as calcium chelate complexes) 3 times / week for three weeks followed by autopsy analysis. All five NAPs used (REP 2055, REP 2139, REP 2163, REP 2165 and REP 2166) had the same sequence composition ($[AC]_{20}$) but each comprised different nucleic acid modifications known to impact the tolerability and stability of oligonucleotides (see Fig. 1). NAP stability in neutral and acidic (modeling intracellular endonuclease activity) human plasma and in duck liver was assessed by fluorescence-HPLC using a fluorescent PNA probe-based hybridization assay. Tolerability was assessed by monitoring weight during treatment, injection site reactivity and findings at autopsy. Antiviral activity was assessed by monitoring serum DHBsAg and anti-DHBpreS (anti-DHBsAg) antibodies by ELISA and serum and liver DHBV DNA by quantitative PCR.

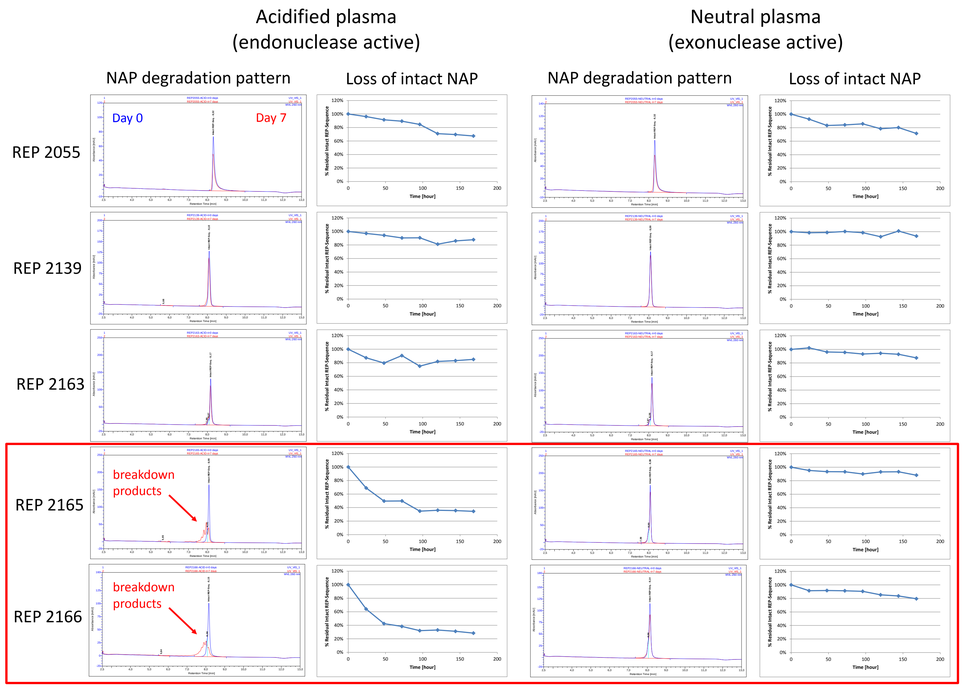
REP 2055	ACACACACACACACACACACACACACACACACACAC
REP 2139	A-CA-CA-CA-CA-CA-CA-CA-CA-CA-CA-CA-CA-CA-CA-CA-CA-CA-CA-CA-C
REP 2163	A-CA-CA-CA-CA-CA-CA-CA-CA-CA-CA-CA-CA-CA-CA-CA-CA-CA-CA-CA-C
REP 2165	A-CA-CA-CA-CA-CA-CA-CA-CA-CA-CA-CA-CA-CA-CA-CA-CA-CA-CA-CA-C
REP 2166	A-CA-CA-CA-CA-CA-CA-CA-CA-CA-CA-CA-CA-CA-CA-CA-CA-CA-CA-CA-C

Sequence Key: DNA (resistant to endonuclease attack)
2'OMe RNA (shielded from endonuclease attack)
2'OH RNA (sensitive to endonuclease attack)

Figure 1. Sequence and chemical modifications of NAPs tested.

RESULTS

Introduction of 2'OH riboadenosines (Fig. 1) accelerated NAP degradation under acidic conditions (Fig. 2) and lead to reduced accumulation of NAPs in duck liver with repeated dosing (Fig. 3 top). No significant changes in weight gain or injection site reactions were observed over the treatment period for any NAP. All NAPs tested reduced serum DHBsAg as compared with the NS-treated control group. The most significant stimulation of anti-DHBpreSAg antibody response was observed in the REP 2139 and REP 2165 groups. Substantial reductions in serum DHBV DNA were found in all NAP groups. Drastic decrease in liver DHBV DNA was observed for four groups treated with REP 2055, REP 2139, REP 2163 and REP 2165. Importantly, liver accumulations for some NAPs that were less resistant to nuclease attack (REP 2055 and REP 2165) were 6-7 fold less at the end of treatment than for REP 2139 yet showed comparable antiviral activity.



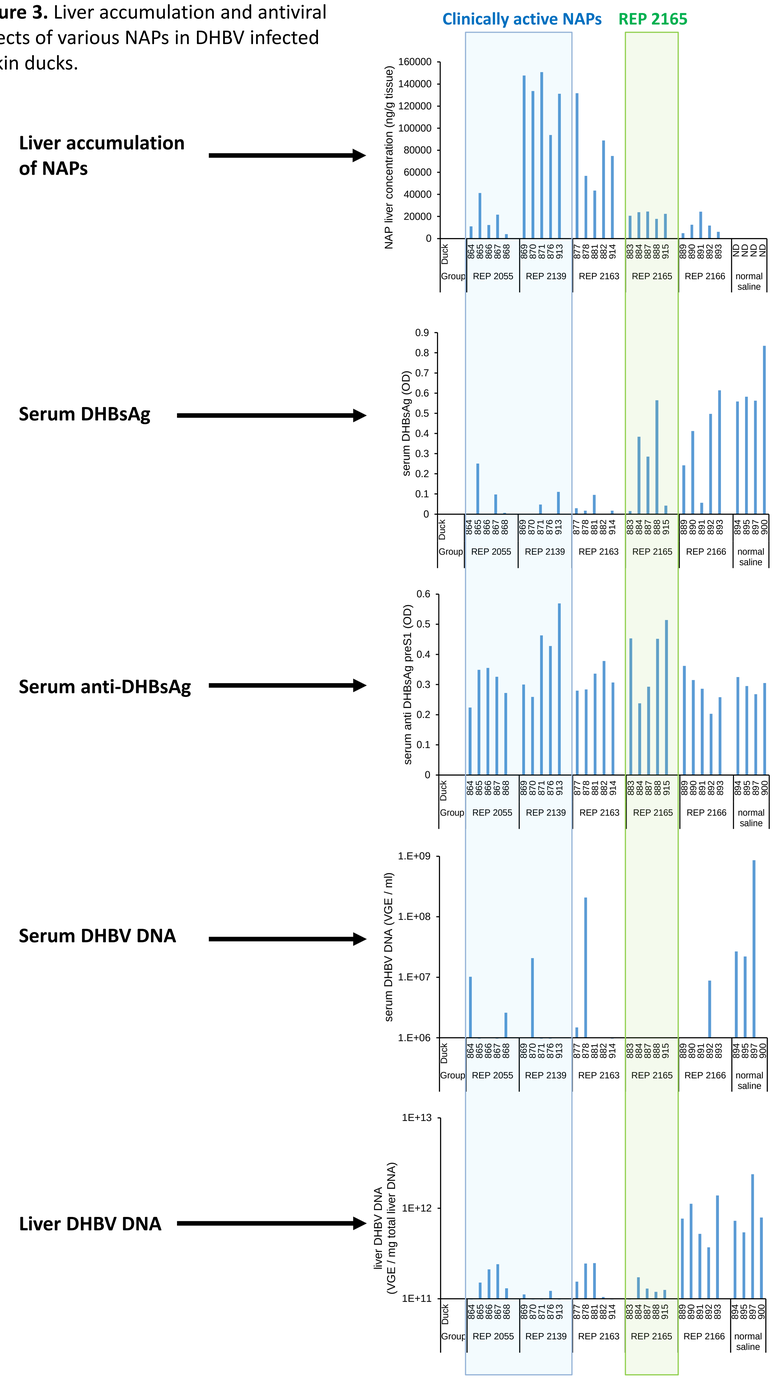
Endonuclease attack at 2'OH riboadenosines accelerates NAP degradation under acidic (i.e. intracellular) conditions.

Figure 2. HPLC analysis of NAP degradation in human plasma over 7 days

CONCLUSIONS

- **The liver accumulation of NAPs can be modulated significantly without affecting their overall antiviral activity.**
- **All NAPs reduced serum DHBsAg and elicit other important antiviral responses in the blood and liver.**
- **The NAP REP 2165 may be of clinical benefit owing to its comparable antiviral activity compared to REP 2139 with significantly lower liver accumulation.**

Figure 3. Liver accumulation and antiviral effects of various NAPs in DHBV infected Pekin ducks.



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