# Modifications of nucleic acid polymers decrease liver accumulation without affecting antiviral activity against HBV

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## BACKGROUND & AIMS

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.

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

- The NAPs used in this study are presented to the right.
- NAP stability in neutral (modelling exonuclease activity) and acidified (modeling intracellular endonuclease activity) human plasma and accumulation in duck liver was assessed by fluorescence-HPLC using a fluorescent PNA probe- based hybridization assay.
- 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 via intraperitoneal injection with 10mg/kg of NAPs (formulated as calcium chelate complexes) 3 times / week for three weeks
- Antiviral activity at end of treatment 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 ACACACACACACACACACACACACACACACACACACAC

REP 2139 A*CA*CA*CA*CA*CA*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*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*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*CA*CA*CA*CA*CA*C

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

## RESULTS

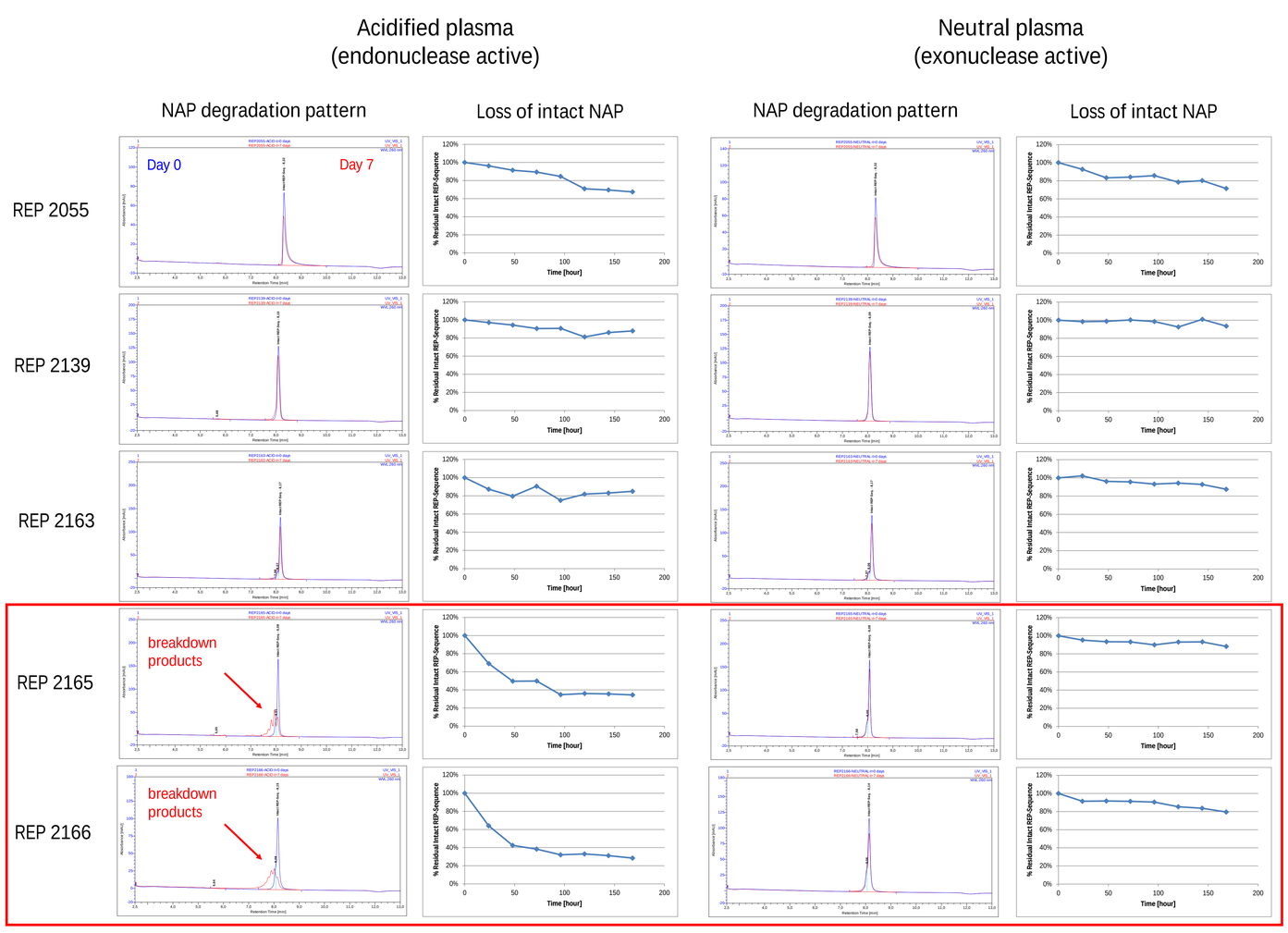


**Figure 1. . HPLC analysis of NAP degradation in human plasma over 7 days.** HPLC analysis of degradation and serum stability of various NAPs in acidified (endonuclease active) and neutral (exonuclease active) human plasma for 7 days (clinical dosing regimen for NAPs). NAPs containing DNA (REP 2055) show moderate loss of the full length NAP over time. NAPs containing all 2'O methylated riboadenosine (REP 2139 and REP 2163) are stable over 7 days of incubation. Introduction of 2'OH riboadenosine at specific locations in REP 2165 and REP 2166 enhances degradation by endonucleases and results in accelerated loss of full length NAPs in acidified human serum over 7 days of incubation.

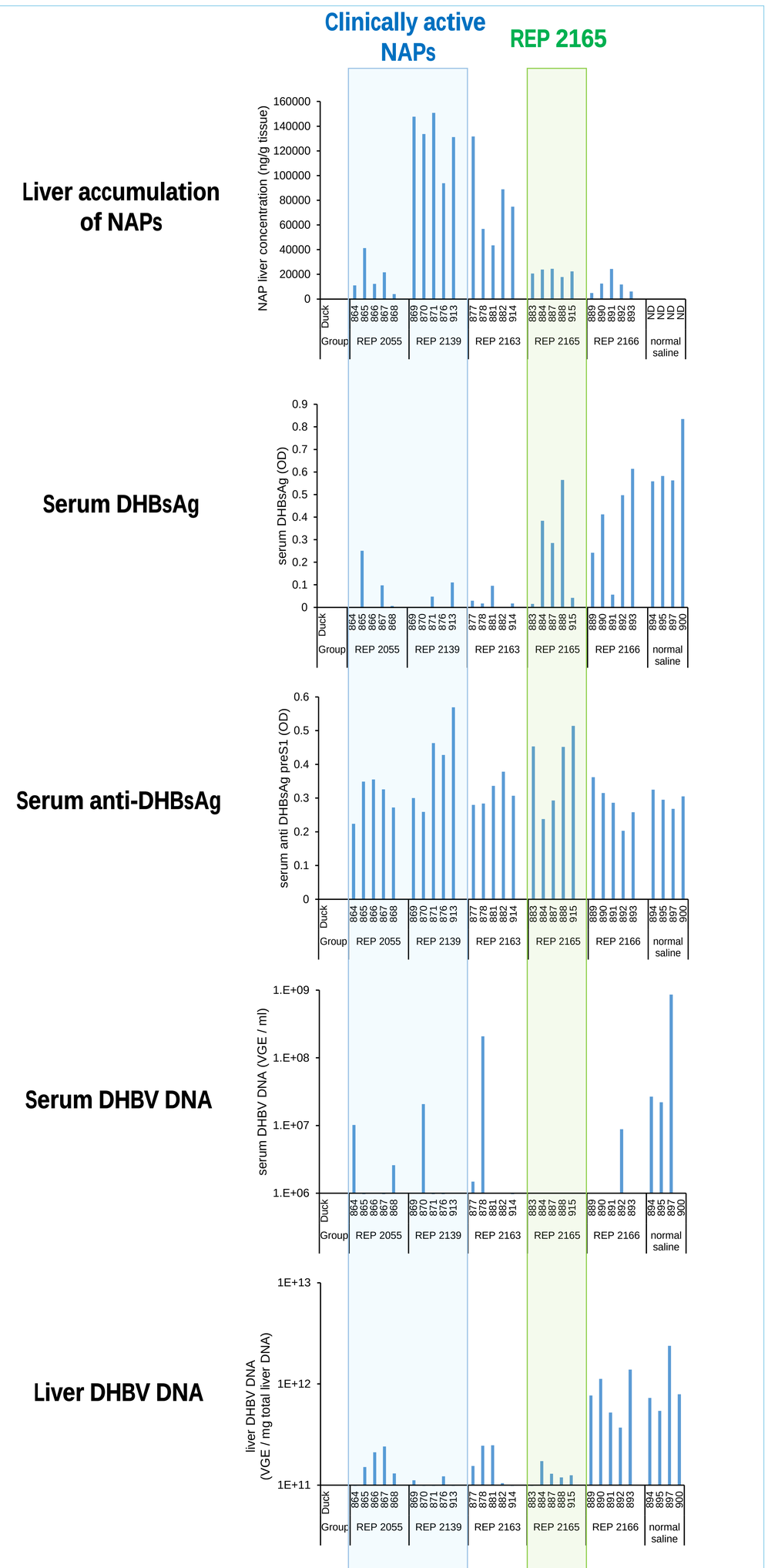


**Figure 2. Liver accumulation and antiviral effects of various NAPs in DHBV infected Pekin ducks.**

## CONCLUSIONS & PERCEPECTIVE

- **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.**

Ø **The NAP REP 2165 may be of clinical benefit owing to its comparable antiviral activity compared to REP 2139 with significantly lower liver accumulation.**

## REFERENCES

1. Noordeen, F., et al, Antimicrob Agents Chemother. 57: 5291-5298.
2. Noordeen, F., et al, Antimicrob Agents Chemother. 57: 5299-5406.

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