



## Activity of nucleic acid polymers in rodent models of HBV infection



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### ABSTRACT

Nucleic acid polymers (NAPs) block the release of HBsAg from infected hepatocytes. These compounds have been previously shown to have the unique ability to eliminate serum surface antigen in DHBV-infected Pekin ducks and achieve multilog reduction of HBsAg or HBsAg loss in patients with chronic HBV infection and HBV/HDV coinfection. In ducks and humans, the blockage of HBsAg release by NAPs occurs by the selective targeting of the assembly and/or secretion of subviral particles (SVPs).

The clinically active NAP species REP 2055 and REP 2139 were investigated in other relevant animal models of HBV infection including woodchucks chronically infected with WHV, HBV transgenic mice and HBV infected SCID-Hu mice. The liver accumulation of REP 2139 in woodchucks following subcutaneous administration was examined and was found to be similar to that observed in mice and ducks. However, in woodchucks, NAP treatment was associated with only mild (36–79% relative to baseline) reductions in WHsAg (4/10 animals) after 3–5 weeks of treatment without changes in serum WHV DNA. In HBV infected SCID-Hu mice, REP 2055 treatment was not associated with any reduction of HBsAg, HBeAg or HBV DNA in the serum after 28 days of treatment. In HBV transgenic mice, no reductions in serum HBsAg were observed with REP 2139 with up to 12 weeks of treatment.

In conclusion, the antiviral effects of NAPs in DHBV infected ducks and patients with chronic HBV infection were weak or absent in woodchuck and mouse models despite similar liver accumulation of NAPs in all these species, suggesting that the mechanisms of SVP assembly and or secretion present in rodent models differs from that in DHBV and chronic HBV infections.

### 1. Introduction

Several models of hepadnaviral infection have been established to investigate the viral replication and pathogenesis of HBV infection in humans (Dandri and Petersen, 2017; Innacone and Guidotti, 2015). Infection of Pekin ducks with duck hepatitis B virus (DHBV), like the infection of woodchucks with woodchuck hepatitis virus (WHV), is a naturally occurring infection which, like WHV-infected woodchucks, establishes a reservoir of cccDNA similar to HBV infection in humans (Cova and Zoulim, 2004; Dandri and Petersen, 2017; Le Mire et al., 2005). Additionally, natural WHV or DHBV infection in woodchucks

and ducks respectively also results in an abundant excess of circulating surface antigen derived from subviral particles (SVP) (Franke et al., 2007; Summers et al., 1978) similar to that observed in HBV infection (Chai et al., 2008). The antiviral responses of various direct acting antiviral agents in the duck model have mirrored their effects in human infection (Nicoll et al., 1998; Foster et al., 2003; Scougall et al., 2012).

The evaluation of the antiviral activity of a variety of investigational antiviral agents have also used many other models, including mice expressing the HBV genome from an integrated transgene (Julander et al., 2002, 2003), from hydrodynamically injected plasmid or adenovirus (Billioud et al., 2016; Martin et al., 2015; Qiu et al., 2016) or

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from injected HBV-infected HepAD38 cells (Feitelson et al., 2007; Schinazi et al., 2012), HBV infected mice with mouse/human chimeric livers (Murakami et al., 2016; Hayashi et al., 2015) or in woodchucks chronically infected with woodchuck hepatitis virus (WHV) (Menne et al., 2008, 2015).

Nucleic acid polymers (NAPs) have a broad spectrum antiviral activity against several enveloped viruses including HBV and hepatitis delta virus (HDV) (Vaillant, 2016). NAPs display potent prophylactic activity in preventing the establishment of persistent DHBV infection in the duck model, an effect which was shown to be driven by a post-entry activity and was effective at doses as low as 1 mg/kg/day (Noordeen et al., 2013a). In established DHBV infection, the post-entry activity of the NAPs REP 2055 and REP 2139 resulted in the reduction of DHBsAg after one week of treatment with clearance of serum DHBsAg achieved as soon as two weeks after the start of treatment (Noordeen et al., 2015; Quinet et al., 2016). This antiviral effect was shown to result from the inhibition of release of DHBsAg from the liver, an effect apparently driven by the inhibition of SVP assembly and/or release (Noordeen et al., 2015). The selective inhibition of SVP assembly and/or secretion by NAPs was recently confirmed *in vitro* in HepG2.2.15 cells (Blanchet et al., 2017). The clearance of DHBsAg during NAP therapy is accompanied by clearance of DHBV DNA from the blood and reduced viral replication in the liver (Roehl et al., 2017), an effect which persists after treatment was withdrawn and was accompanied by the absence of detectable DHBcAg, DHBsAg, and multilog reduction of cccDNA in the liver (Noordeen et al., 2015; Quinet et al., 2016).

The antiviral effects of NAPs observed in DHBV infection *in vivo* have mirrored those observed in subsequent clinical trials in HBV infection. Both REP 2055 and REP 2139 rapidly reduce or clear HBsAg in patients with HBeAg-positive and HBeAg-negative chronic HBV infection and in patients with HBV/HDV co-infection with weekly doses as low as ~3 mg/kg/week (Al-Mahtab et al., 2016; Bazinet et al., 2017a, 2017b). HBsAg clearance is accompanied by seroconversion of HBeAg (in HBeAg positive patients), unmasking of anti-HBs and clearance of HBV DNA. Notably NAPs also display a direct antiviral activity against HDV distinct from their effects on SVPs (Bazinet et al., 2017a). Most importantly, the clearance of HBsAg in patients has resulted in dramatically improved antiviral response to immunotherapy, with its use in the absence of serum HBsAg associated with dramatic increases in anti-HBs, the onset of therapeutic liver flares and the establishment of functional control of HBV and HDV infection persisting after treatment has been withdrawn.

NAPs are phosphorothioate oligonucleotides, a chemical class which enter hepatocytes and efficiently accumulate in the liver of mice and non-human primate species (Geary et al., 2015a). These conserved pharmacokinetic behaviours, also observed for NAPs in these species, are also conserved in Pekin ducks (Roehl et al., 2017). This study investigated the pharmacological behaviour of NAPs in woodchucks and their antiviral activity in other well established rodent models to better understand the mechanism of action of NAPs in hepadnaviral replication and secretion.

## 2. Material and methods

### 2.1. NAP synthesis and formulation

REP 2055 and REP 2139 are 40mer phosphorothioate oligonucleotides with the sequences (dAdC)<sub>20</sub> and (2'OMeA, 2'OMe-5-MeC)<sub>20</sub>, respectively (Vaillant, 2016). REP 2031 is a 40mer phosphorothioate oligonucleotide with the sequence dC40 (Noordeen et al., 2013a). REP 2055 and REP 2139 were prepared under cGMP as described previously (Al-Mahtab et al., 2016). REP 2031 was prepared under cGMP-like conditions (Noordeen et al., 2013a). REP 2055 and REP 2031 were

prepared as stocks in normal saline prior to administration. REP 2139 was prepared either in normal saline or as a calcium chelate complex (REP 2139-Ca, Al-Mahtab et al., 2016) prior to administration.

### 2.2. Tissue distribution of NAPs in woodchucks

Woodchucks without WHV infection (n = 3) were purchased from the Institute of Experimental Animal Science, CAMS (Beijing, China) and maintained according to the guidelines of the animal facility of Huazhong University of Science and Technology in Wuhan. Woodchucks were dosed with 16 mg/kg of REP 2139-Ca via bolus subcutaneous (s.c.) injection. One day following injection, serum and tissue samples were obtained during sacrifice and held at –80 °C until analysis. The concentrations of REP 2139 in serum, liver and kidney were determined by a previously validated fluorescence-HPLC based method (Roehl et al., 2017). Specificity of NAP detection was verified on tissues matrices from an untreated animal (data not shown).

### 2.3. Treatment and monitoring of WHV infected woodchucks

Chronically woodchuck hepatitis virus (WHV) infected woodchucks (*Marmota monax*) were purchased from North Eastern Wildlife (Ithaca, New York, United States of America) and maintained according to the guidelines of the animal facility of the University Hospital Essen. For blood sampling, woodchucks were anesthetized by intramuscular injection with 4 ml of 10% Ketamine mixed with 1 ml 2% of xylazine (Ceva, Tiergesundheit, Germany) and blood was taken from the hind limb vein (vena saphena).

In the first experiment REP 2055 and REP 2139 (each n = 2) was dosed at 10 mg/kg three times per week s.c. for three weeks. Sampling at baseline (week 0) weeks 1, 2 and on week 4 just after completion of treatment. In the second experiment, REP 2139-Ca (n = 6), dosed at ~15 mg/kg was administered three times per week s.c. for five weeks. Sampling was performed at baseline, week 1 and week 5.

Serum was frozen at –20 °C until processing. WHV DNA was quantified by real-time qPCR using WHc-specific primers wc1 and wc149s (Meng et al., 2014) (wc1; sense primer: TGGGGCCATGGATA-TAGATCCTTA; WC149S; anti-sense primer: AAGATCTCTAAA TGACTGTATGTTCCG). WHsAg was monitored by electro-immunodiffusion (Laurell electrophoresis) as described previously (Gerlich et al., 2004). In brief, 10 µl of the woodchuck sera (or suitable dilutions thereof) were applied to 3 mm holes on glass slides that were covered with 6 ml of 0.6% agarose and, containing 80 µl of rabbit polyclonal anti-WHs antiserum and run for 16 h at 5 mA per slide. Using a standardized serum and purified WHsAg from WHV-infected woodchucks, the length of the precipitation arc was converted into mg WHsAg/ml.

### 2.4. Treatment and monitoring of HBV infected SCID-Hu chimeric mice

The use of SCID-Hu mice and the experimental procedures used to treat these animals was approved by the Animal Ethics Committee of Phoenix Bio (Resolution No. 0973). Male uPA<sup>+/+</sup>/SCID-Hu mice were prepared and infected with HBV genotype D as previously described (Tuge et al., 2005; Utoh et al., 2008) and the viability of the liver chimera was monitored during the treatment by serum levels of human albumin (LX Reagent “Eiken” Alb II, Eiken Chemical Co. Ltd.). Animals were 21–24 weeks of age and were verified to have well established HBV infection at the start of treatment by assessment of viremia and received 28 days of treatment with REP 2055 or REP 2031 (10 mg/kg/day via intraperitoneal injection (i.p.)) or entecavir (ETV) (0.03 mg/kg/day via oral gavage). Control animals received volume matched, daily i.p. administration of normal saline. Antiviral effects during treatment

were assessed by monitoring HBsAg (Abbott Architect quantitative), HBeAg (Abbott Architect) and HBV DNA (in-house qPCR) in blood taken before administration every 3 days during treatment.

### 2.5. Treatment and monitoring of HBV transgenic mice

Studies in transgenic mice were conducted in accordance with the approval of the Institutional Animal Care and Use Committee of Utah State University, which approval has an expiration date of 30 June 2017. Two strains of adult homozygous male and female transgenic HBV mice were used: one obtained from Dr. Frank Chisari (Guidotti et al., 1995), and another from Dr. Patricia Marion (Marion et al., 2003). Whole blood was collected by submandibular cheek bleeding or cardiac exsanguination at necropsy, and sera was collected and stored at  $-80^{\circ}\text{C}$  until use. Mice were pre-screened for active HBV infection and randomized into treatment and control groups (where applicable) using HBsAg prior to treatment. Animals were treated for 3 or 12 weeks with REP 2139-Ca (10 mg/kg, 3 times per week via i.p. injection) and HBsAg was monitored regularly before and during treatment by quantitative ELISA (Immuno Diagnostics, Foster City, CA).

## 3. Results

### 3.1. Effects of NAPs in chronically WHV-infected woodchucks

The uptake of NAPs into hepatocytes is a prerequisite for their effects on viral replication, therefore, the biodistribution of REP 2139 24 h following s.c. injection in woodchucks was examined and demonstrated liver accumulation in this rodent species (Fig. 1). S.c. administered REP 2139 was observed to accumulate in the woodchuck liver and kidney with concentrations of  $106.4 \pm 49.5$  and  $172.6 \pm 5.01$   $\mu\text{g/g}$  tissue respectively, consistent with the behaviour of parenterally administered REP 2139 in mice, ducks and cynomolgus monkeys (Roehl et al., 2017). The plasma concentration of REP 2139 was significantly lower ( $2.6 \pm 0.97$   $\mu\text{g/mL}$ ) at 24 h post administration, consistent with the known plasma clearance behaviour of subcutaneously administered phosphorothioate oligonucleotides and other NAPs (Leeds et al., 2000; data not shown).

The first experiment in woodchucks consisted of testing the two clinically active NAP species, REP 2055 and REP 2139, each in two chronically WHV-infected animals. Both NAPs were administered three times per week at 10 mg/kg for three weeks and the effects on WHsAg, WHV DNA and AST were evaluated during treatment (Fig. 2A). WHsAg

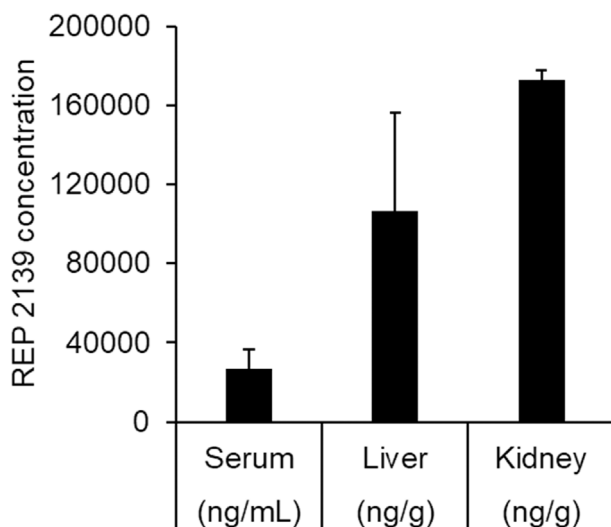


Fig. 1. Biodistribution of REP 2139 in serum, liver and kidney of woodchucks, 24 h after a bolus s.c. injection of 10 mg/kg of REP 2139-Ca. Plotted values are mean  $\pm$  standard deviation ( $n = 3$ ).

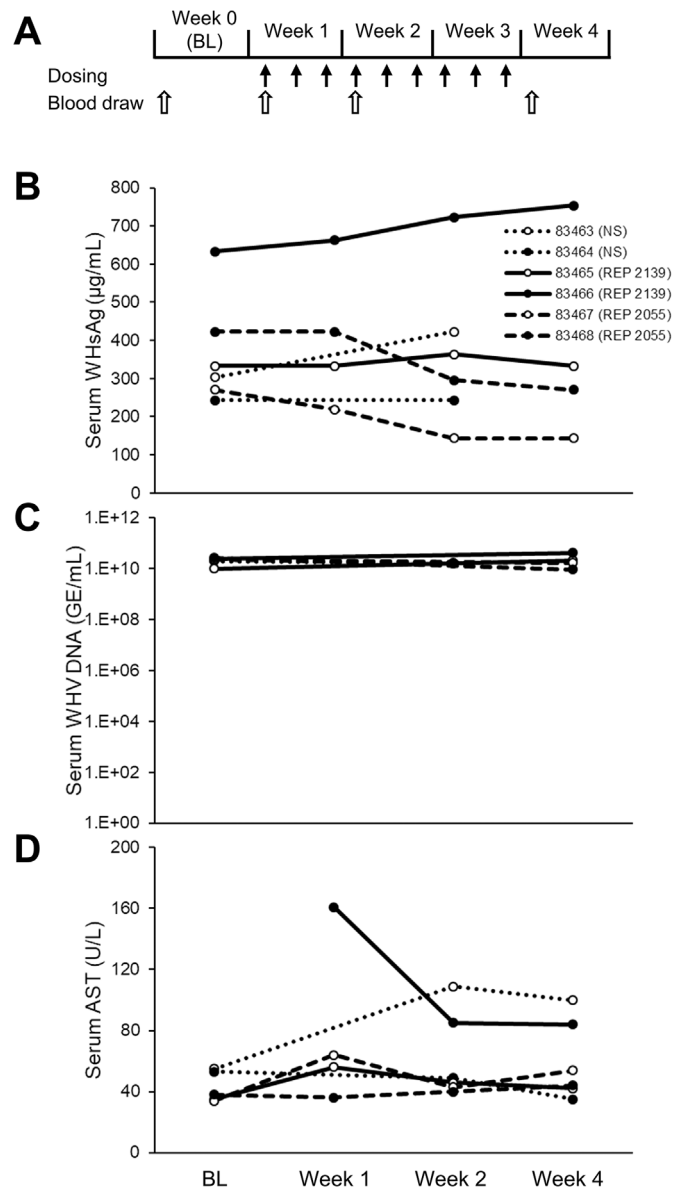
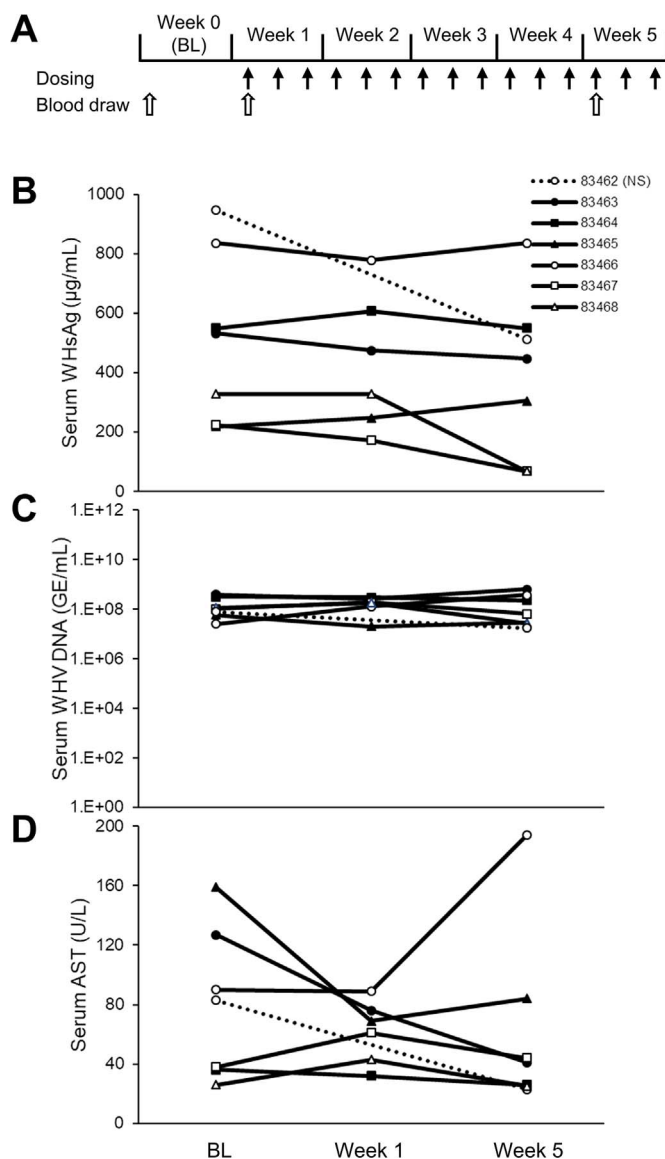


Fig. 2. Antiviral effects of REP 2055 and REP 2139 in woodchucks with chronic WHV infection ( $n = 4$ ). Experimental design is indicated in (A). Changes during treatment in serum WHsAg (B), WHV DNA (C) and AST (D) are presented. BL = baseline.

reductions of 36 and 47% from baseline to week 3 were observed in the two animals receiving REP 2055 whereas no reduction in WHsAg occurred in the two animals receiving REP 2139 (Fig. 2B). No changes in WHV DNA or AST were observed (Fig. 2C and D) except for AST decline observed in one animal during REP 2139 treatment.

The second experiment used the same REP 2139-Ca formulation previously used in clinical trials (Al-Mahtab et al., 2016; Bazinet et al., 2017a) with the same dosing duration and frequency as in the first woodchuck experiment but with higher dose ( $\sim 15$  mg/kg) and longer exposure (Fig. 3A) in six WHV infected animals. In two animals, WHsAg reductions of 69 and 79% from baseline were observed animals 83467 and 83468 but no WHsAg reduction were observed in the other four animals (Fig. 3B). No changes in WHV DNA (Fig. 3C) or AST (Fig. 3D) were observed, except in one woodchuck (83466) receiving REP 2139-Ca with AST elevation at week 5, which was not accompanied by any antiviral response. WHsAg reduction was observed in the control animal (83462) in this experiment, a rare effect also reported in other studies (Meng et al., 2016).



**Fig. 3.** Antiviral effects of REP 2139-Ca in woodchucks with chronic WHV infection ( $n = 6$ ). Experimental design is indicated in (A). Changes during treatment in serum WHsAg (B), WHV DNA (C) and AST (D) are presented. BL = baseline.

### 3.2. Effects of NAPs in HBV infected SCID-Hu mice

The NAPs REP 2055 and REP 2031 were tested in SCID-Hu mice with pre-established HBV (genotype D) infection. REP 2031 is a control NAP which has shown to have a mild entry-inhibition activity *in vitro* against HBV and DHBV but no post-entry activity in DHBV and negligible antiviral effect against DHBV infection *in vivo* (Guillot et al., 2017; Noordeen et al., 2013a, 2013b). Dosing in these mice consisted of daily administration of 10 mg/kg REP 2055 or REP 2031 daily for 28 days. Separate groups of mice were treated with entecavir (ETV) as a positive control or normal saline as a negative control. Animal weights were not altered in any treatment group throughout dosing (Fig. 4A) and the human chimeric livers in these mice were stable, as evidenced by consistent production of human albumin throughout treatment (Fig. 4B). However, neither REP 2055 nor REP 2031 had any effect on HBsAg or HBeAg (Fig. 4C and D) or HBV DNA (Fig. 4E). Animals in the ETV group experienced mild reductions in both HBsAg (0.45log) and

HBeAg (0.31log) and more substantial reductions in viremia (2.68log) compared to mice in the normal saline group.

### 3.3. Effects of NAPs in HBV transgenic mice

The antiviral effects of REP 2139-Ca were assessed in a preliminary experiment in two different strains of transgenic mice, the “Chisari” strain ( $n = 6$ ) and the “PAMF” strain ( $n = 7$ ). In this experiment, the stability of serum HBsAg levels was monitored for 2 weeks prior to dosing, which consisted of 10 mg/kg every other day for 16 days. During this treatment, no reduction of serum HBsAg was observed (Fig. 5A).

Since the PAMF mice produced significantly higher levels of circulating HBsAg, a second experiment was conducted assessing the effects of 12 weeks of therapy with REP 2139-Ca (10 mg/kg, 3 times per week) in 11 animals with a matched control group receiving normal saline. In this second, long term dosing experiment, no reduction in HBsAg was observed and HBsAg levels were similar to those in normal saline treated animals (Fig. 5B).

## 4. Discussion

The pharmacokinetic and biodistribution properties of NAPs are similar in mice, ducks and non-human primates and consists of clearance of NAPs from the plasma with accumulation primarily in the kidney and liver (Roehl et al., 2017). The accumulation of NAPs in the liver and kidney after subcutaneous administration of REP 2139 in the woodchuck as demonstrated in this study is consistent with the pharmacokinetic behaviour of NAPs observed in other animal models. In preclinical studies of the antiviral effects of NAPs in DHBV infected Pekin ducks, the onset of DHBsAg reduction with NAPs is reproducibly rapid, with clearance occurring within one week of daily 10 mg/kg dosing (Noordeen et al., 2013a, 2015). This antiviral effect is retained in ducks with NAP dosing as low as 1 mg/kg/day (Noordeen et al., 2013a). In contrast, reductions in WHsAg during NAP treatment of chronic WHV infected woodchucks were very mild, ranging from 36 to 79% in both experiments, was slow in onset and only occurred in 4/10 treated woodchucks. These reductions in WHsAg were not accompanied by any reduction in WHV DNA.

In mice, antiviral activity of NAPs in the liver against CMV is readily achieved in mice with daily i.p. or s.c. dosing as low as 2 mg/kg (Cardin et al., 2009) and in HCV infected SCID-Hu mice with i.p. dosing every other day at 10 mg/kg (Matsumura et al., 2009). However, neither REP 2055 nor REP 2139 had any effect on antigenemia or viremia in two different mouse models of HBV infection using dosing regimens demonstrated to achieve abundant levels of NAPs in the liver in this species (Roehl et al., 2017). Similar dosing regimens have resulted in rapid surface antigen clearance in DHBV infected ducks or have demonstrated antiviral activity against other hepatotropic viruses in infected normal mice or HCV infected SCID-Hu mice (Cardin et al., 2009; Matsumura et al., 2009). The weak or absent surface antigen reductions with REP 2055 and/or REP 2139 in woodchucks and mice is striking as both these NAPs have been shown to reliably achieve rapid clearance of DHBsAg in DHBV infection *in vivo* (Noordeen et al., 2015; Quinet et al., 2016) and HBsAg in chronic HBV infection in diverse patient populations in several clinical trials (Al-Mahtab et al., 2016; Bazinet et al., 2017a, 2017b). This lack of efficacy of NAPs in rodent models cannot be due to a pharmacokinetic or dosing level issue as described above. Moreover, it is well documented that the systemic exposure of phosphorothioate oligonucleotides (PS-ONs) in rodents and non-human primates is similar with any route of parenteral administration. Whether administered via s.c, i.p or intravenous routes (i.v.) routes, this class of compounds are rapidly cleared from the circulation and



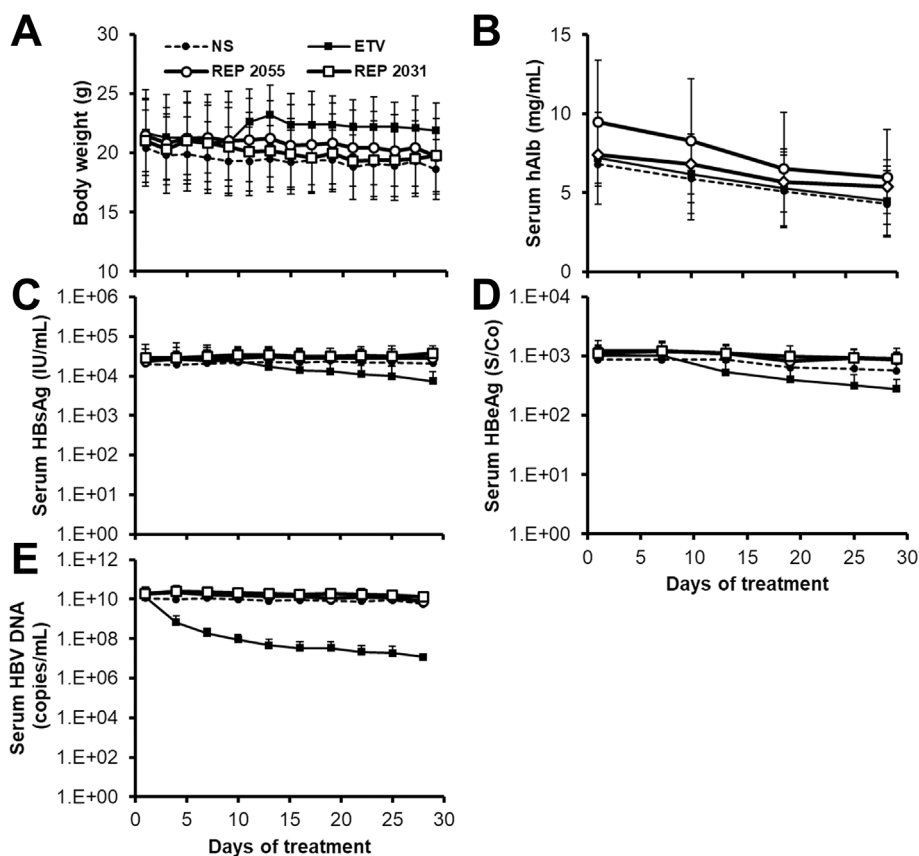


Fig. 4. Antiviral effects of entecavir, REP 2055 and REP 2031 in HBV infected SCID-Hu mice. Changes in body weight (A), serum human albumin (B), HBsAg (C), HBeAg (D) and HBV DNA (E) are presented. Plotted values are mean  $\pm$  standard deviation (n = 5).

accumulate primarily in the kidney and liver and to a lesser extent in the spleen, lungs and other organs. These common behaviours have been exhaustively confirmed with numerous different species of PS-ONs in mice, rats, non-human primates and humans (Yu et al., 2007; Levin et al., 2007) and have been previously verified for NAPs in mice and non-human primates (Roehl et al., 2017). As such, achievement of pharmacological activity in the liver with other PS-ONs (antisense oligonucleotides) in human patients is similar whether these compounds are administered i.v. or s.c. (Graham et al., 2013; Janssen et al., 2013; Geary et al., 2015b).

The verification of this conserved pharmacological behaviour of NAPs in woodchucks with parenteral administration in the current study (rapid clearance from the circulation with concomitant accumulation in the kidney and liver) clearly demonstrates that the pharmacokinetic behaviour of NAPs (i.e. PS-ONs) in woodchucks is (as expected) the same as in other rodent species and non-human primates. Further, s.c. administered NAPs clearly result in their accumulation in the liver of woodchucks (an effect which would clearly be no different with i.v. administered NAPs) with little or no antiviral effect. As such, the discrepancy between the antiviral effects of NAPs in human HBV infection and rodent models of HBV infection is not due to different routes of administration used. Instead, these differences may be related to the absence in rodents of some element involved in the secretion of SVPs present in chronic HBV infection and in DHBV infected ducks.

The molecular mechanisms of SVP assembly and secretion are currently unknown and the manner in which the assembly and or secretion of SVPs is inhibited by NAPs is still under investigation. However, the current data in HepG2.2.15 cells *in vitro*, in DHBV infection *in vivo* and the close correlation of these antiviral effects with those in HBV infected patients clearly establish that NAPs inhibit SVP morphogenesis and or secretion in the human disease. This novel antiviral mechanism

is highly relevant for therapeutic impact in human patients. As such, the lack of activity of NAPs in rodent models of HBV infection may provide clues as to how NAPs are acting.

SVPs from patients with chronic HBV infection contain serum components and are closely related to HDL in both lipid and cholesterol composition (Neurath et al., 1974; Burrell, 1975; Gavilanes et al., 1982), suggesting that the assembly and or secretion of SVPs may rely on some aspect of HDL lipid metabolism. More recent studies also support a role for lipid/HDL metabolism in SVP assembly/trafficking (Lin et al., 2002; Satoh et al., 2000). HDL are remarkably similar in avian species and humans (Kruski and Scanu, 1975), however the cholesteryl ester transfer protein (CETP), an important enzyme which shuttles cholesterol esters from HDL to LDL and VLDL in humans, is also abundant in avian species but is absent in rodent species (Guyard-Dangremont et al., 1998). This important biochemical difference in rodents results in the bulk of plasma cholesterol being carried in HDL instead of LDL and VLDL as in avian species and humans (Camus et al., 1983). The fact that SVPs are highly similar to HDL, combined with the correlation between the lack of surface antigen response to NAPs in rodent species versus ducks and humans and the differences between in HDL metabolism between rodents and avians/humans suggests that HDL metabolism may be important in SVP production. Moreover, NAPs may somehow target an aspect of host HDL metabolism involved in SVP morphogenesis and or secretion not present in rodent species. Additional investigation will be required to validate this hypothesis.

The SCID-Hu model has an advantage over other rodent models in that the human chimeric liver present in these mice supports the infection and propagation of HBV infection with inoculation of virus derived from human sera. Importantly, the establishment of liver chimeras in SCID-Hu mice with a replacement index of < 70% is accompanied by the establishment of a human lipoprotein profile and the

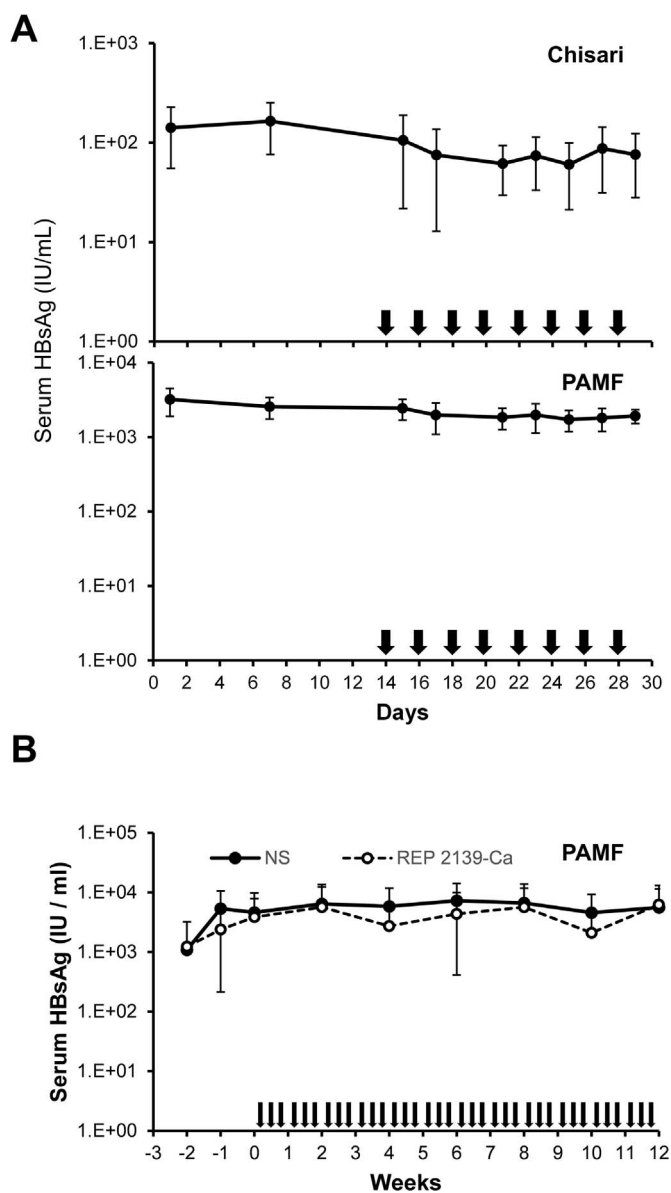


Fig. 5. Antiviral effects of REP 2139-Ca in HBV transgenic mice. A) changes in serum HBsAg levels in “Chisari” (top) and “PAMF” (bottom) HBV transgenic mice during two weeks of treatment with REP 2139-Ca. B) changes in serum HBsAg levels in PAMF HBV transgenic mice during 12 weeks of treatment with REP 2139-Ca. Arrows indicate treatment days. Plotted values are mean  $\pm$  standard deviation (n = 6 [Chisari] and n = 7 [PAMF] in (A), n = 11 in (B)).

presence of CETP (Steenbergen et al., 2010). Human albumin expression levels in the SCID-Hu mice used in this study indicated a replacement index of > 70% but NAPs were still ineffective in this model, despite the fact that NAPs are active against HCV infection in these mice (Matsumura et al., 2009). In this model, NAPs were active in blocking HCV entry into the liver but had no antiviral effect against established HCV infection. However, the restriction to entry inhibitory activity observed *in vivo* is consistent with entry inhibitory properties of NAPs against HCV described in this study and as the activity of NAPs in patients with HCV infection is unknown, lack of post-entry activity in this setting is difficult to interpret.

On the other hand, it has been well established that the antiviral effect of NAPs is to work at post entry to block the release of subviral particles (Blanchet et al., 2017). NAPs with no entry activity (i.e. REP 2139, Guillot et al., 2017) are fully active in ducks and humans (Bazinet et al., 2017a, 2017b; Quinet et al., 2016) and a control NAP (REP 2031)

capable of blocking entry (Guillot et al., 2017) but having no post-entry effect (Noordeen et al., 2013b) has no activity *in vivo* the duck model (Noordeen et al., 2013a) and is also inactive in Scid-Hu mice as shown in the current study. As such, the lack of activity of NAPs in the therapeutic setting in rodents (where post-entry effects are best evaluated) clearly indicates the absence of antiviral activity whether administered before or after infection.

The inactivity of NAPs in HBV infected SCID-Hu mice appears at odds with the presence of HBV infected human hepatocytes in this model. Moreover, the selective targeting of SVP assembly and or secretion recently demonstrated in HepG2.2.15 cells is achieved in the absence of any human supplements (Blanchet et al., 2017), demonstrating that the antiviral activity of NAPs is not dependent on factors from other human tissues. Therefore, the lack of other human cell types in the SCID-Hu model is an unlikely reason for the lack of activity of NAPs. However, the human regions of the chimeric livers in SCID-Hu mice display prominent steatosis, lack of sinusoids and the absence of bile canalicular formation between human and mouse hepatocytes (Peterson et al., 2010), indicating altered lipid metabolism and a potentially incomplete biosynthetic functioning of these hepatocytes. These defects may affect SVP morphogenesis and or trafficking in this model despite the presence of HBV infected human hepatocytes.

## 5. Conclusions

Several studies with investigational agents against HBV infection that target HBV replication upstream of SVP assembly and or secretion have resulted in HBsAg reductions in rodent models (Billioud et al., 2016; Martin et al., 2015; Menne et al., 2015), indicating that rodent-based models of HBV infection have had and will continue to have an important utility in evaluating the antiviral effects of investigational agents against HBV infection. However, these studies suggest that the aspects of SVP assembly and or secretion targeted by NAPs in patients with chronic HBV infection are not well modeled in currently available rodent-based models of HBV infection. This possibility should be considered when interpreting the antiviral effects (especially HBsAg reduction) of new investigational agents directly targeting SVP assembly and or secretion in these models.

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## Glossary

CMV	cytomegalovirus
DHBsAg	duck hepatitis B virus surface antigen
DHBV	duck hepatitis B virus
HBsAg	hepatitis B virus surface antigen
HBV	hepatitis B virus
HCV	hepatitis C virus
HDL	high density lipoprotein
HDV	hepatitis delta virus
LDL	low density lipoprotein
WHsAg	woodchuck hepatitis virus surface antigen
WHV	woodchuck hepatitis virus
SCID-Hu	[uPA <sup>+/+</sup> ; B6SJL-TgN(Alb1Plau)144Bri, SCID: C.B-17/Icr-scid/scid Jcl] mice containing human hepatocytes
VLDL	very low density lipoprotein

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