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Inhibition of Hepatitis B Virus in Mice by Cationic Liposome/RNA Oligonucleotide Complex*

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Abstract We investigated the inhibitory effect of modified antisense RNA oligonucleotides and cationic liposome-RNA complexes on the repression of hepatitis B virus (HBV) replication and expression. ELISA and quantitative Real-time PCR analysis showed that HBV replication and antigens expression both in pHBV1.3 transduced HepG2 and HepG2.2.15 cells were reduced after treatment with antisense RNA oligonucleotides P-2987, X-60 and X-519. Subsequently, the antisense RNA oligonucleotides and control RNA oligonucleotides were injected *via* the tail vein into HBV transgenic mice or hydrodynamically injected mice. In the HBV transgenic mice, with the treatment of X-519, HBV pregenomic RNA in the liver decreased by 81%. Cationic liposome further increased the inhibition effectory to 91%. But no significant differences were observed for HBV antigens and HBV DNA copy number in the sera. In acute HBV infection mouse model by hydrodynamic injection, ELISA and quantitative real-time PCR analysis showed that X-519 significantly repressed HBV replication, as measured by HBV pregenomic RNA, antigens expression, and presence of HBV DNA in the sera. Taken together, the synthesized antisense RNA oligonucleotide X-519 repressed HBV replication and antigens expression *in vitro* and *in vivo*.

Key words hepatitis B virus, antisense RNA oligonucleotides, HBV transgenic mice, hydrodynamic injection, cationic liposome-RNA complex
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Hepatitis B virus (HBV) is the prototypical member of the Hepadnaviridae (hepatotropic DNA virus) family. HBV virions are double-shelled particles, with an outer lipoprotein envelope that contains three related envelope glycoproteins. The HBV genome has only four open reading frames (ORFs) [1]. The P coding region of the genome is specific for viral polymerase, a multifunctional enzyme involved in DNA replication and RNA encapsidation. The precore-core open reading frame encodes hepatitis B virus e antigen (HBeAg) and hepatitis B virus core antigen (HBcAg). The presurface-surface region encodes the three types of hepatitis B virus surface antigen (HBsAg). The X region encodes the viral X protein (HBxAg). The viral RNAs transcribed from these ORFs include pregenomic RNA, which serves as the template for reverse transcription during the steps of replication, as well as three subgenomic mRNAs necessary for the translation of the envelope proteins and the mRNA for the X protein. The pregenomic RNA serves as an mRNA both for the viral core protein and for the viral polymerase.

HBV infection, which can cause acute or chronic hepatitis, cirrhosis, and hepatocellular carcinoma, is a common health burden around the world, especially in developing countries such as China. Despite the introduction of HBV vaccination programs in the last decade, hepatitis B remains a largely disseminated disease, with an estimated 350 million people chronically infected around the world, and over 2 billion people who have already been infected with the virus. It is estimated that between 1 and 2 million

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people die annually as a consequence of the infection[2]. Current treatment strategies include interferon or nucleoside analogues, as well as prevention *via* vaccination. In many patients, a flare of liver injury occurs during administration of interferon alpha, and treatment with interferon alpha is generally contraindicated in very advanced liver disease. The limitation of lamivudine mono-therapy is the development of drug resistance, which is largely mediated by point mutations[2-4].

Antisense RNA (asRNA) is a single-stranded RNA that can bind to an mRNA by complementary base pairing and is involved in regulation of specific gene expression through mRNA silencing[5]. 2'-O-methyl RNAs, which carry a methyl group at the 2'-OH residue of the ribose molecule, display the same behavior as DNA but are protected against nuclease degradation. They have been used in the inhibition of various viral pathogens and are considered a new generation of drugs [6]. Cationic liposome have been used as an oligonucleotide delivery tool *in vitro* and *in vivo* to transport RNA to targeted sites and delay degradation of the RNA[7].

The host range of HBV is limited to humans and chimpanzees. Thus, to date, there is no suitable HBV-sensitive model for research, which is currently limited to cell lines and transgenic mice carrying the HBV genome [4, 8]. Transgenic mice [9] carrying 1.3 copies of the complete genome of HBV subtype ayw display high levels of HBsAg expression and HBV DNA copy number in sera. HBV pregenomic RNA is also highly expressed in the extract of mouse liver tissue. The mouse model of acute HBV infection, which is based on hydrodynamic injection [10] of plasmids carrying 1.3 copies of the HBV genome, is also used to detect the repression of HBV by asRNA oligonucleotides[11]. In this report, we designed and modified three asRNA oligonucleotides complementarily targeting the HBV viral mRNAs. To enhance the delivery efficiency and the stability of the asRNA oligonucleotides *in vivo*, we combined the synthesized asRNAs with cationic liposome to investigate the inhibition effectory in mouse models of HBV infection.

1 Materials and methods

1.1 RNA oligos

The 2'-O-methyl asRNA oligonucleotides with a 3'-end butanol modification [12] targeted to HBV viral mRNAs were synthesized and high-pressure liquid chromatography (HPLC) purified at Oligos Etc Inc., Oregon.

The sequences of the antisense molecules are: UUCACGGUGGUCUCCAUGCG (P2987); CUAGCAGCCAUGGAAACG (X60); and GAGAUGAUUAGGCAGAGGUG (X519).

A randomized RNA oligonucleotide was also designed as a negative control (AGACCTCTCATAGCAGCTGAT).

1.2 Animals

Female BALB/c mice (6- to 7-weeks-old) were purchased from the Infectious Disease Center of the 458 Hospital of the People's Liberation Army, China, and housed in standard pathogen-free conditions. The mice were HBV genome (ayw subtype)-integrated and presented high levels of HBsAg and HBV DNA in their sera. The animal experiments were performed within the guidelines of regulation for the use of experimental animals of Chinese Academy of Sciences.

1.3 Cell culture and transfection

HepG2.2.15 cells, a derivative of the human HepG2 hepatoma cell line stably transformed with the HBV genome, were maintained in DMEM supplemented with 10% FBS, 400 mg/L G418, 100 mg/L streptomycin, and 100 U/ml penicillin at 37℃ in a humidified atmosphere containing 5% CO_2. Fifty thousand cells per well were seeded into 6-well plates and transfected with asRNAs (2 μmol/L) or co-transfected with asRNA (2 μmol/L) and pHBV1.3 (1 μg/well) using Lipofectamine 2000 (Invitrogen). The cells and the medium were harvested 72 h post-transfection for analysis.

1.4 RNA oligonucleotide treatment *in vivo*

In all of the experiments, the asRNA oligonucleotides were delivered through tail vein injection at a dose of 100 μg (volume is 0.1 ml) per injection (～5 mg/kg) every 2 days for 2 weeks. Mice were weighed every day during treatment. The day after the last injection, mice were sacrificed, and samples of sera and livers were collected as described.

1.5 Cationic liposome and liposome-RNA complexes

Cationic liposome (DMRIE-C) were purchased from Invitrogen. During the liposome-RNA complex treatment, the mixture of cationic liposome and

asRNA oligonucleotides was delivered through the tail vein at a 1∶4 ratio every 2 days for 2 weeks. The liposome-RNA complexes were freshly mixed and incubated for 30 min just before injection. The size, polydispersity (PDI), and zeta-potential of the liposome-RNA complexes were measured using a zetasizer (nano-ZS, MALVERN, National Center for NanoScience and Technology, China).

1.6 Mouse model of acute HBV infection using hydrodynamic injection

The DNA construct pHBV1.3 (ayw subtype, provided by Prof. WANG Fu-Sheng), which constructs 1.3 copies of HBV genomic DNA and contains HBV intrinsic promoter necessary for pgRNA transcription, was intravenously injected into BALB/c mice under hydrodynamic conditions to transfect hepatocytes *in vivo*[13]. Briefly, 16 μg of pHBV1.3 plasmid dissolved in a volume of 0.9% saline equivalent to 8% of the body mass of the mouse (*e.g.*, 1.6 ml for mouse of 20 g body mass) was rapidly injected into the tail vein within 5～8 s. The mice were bled, and the sera were separated and examined for HBsAg, HBeAg, and HBV DNA.

1.7 Antigens and HBV DNA in sera

ELISAs were performed to relatively quantify HBsAg (or HBeAg) secreted into the cell medium or the sera using Diagnostic Kits for Hepatitis B s Antigen (or e Antigen; Kehua Bioengineering Co. Ltd.).

HBV DNA copy numbers were quantitated by fluorescent Real-time PCR according to the manufacturer's instructions (Daan Gene Co. Ltd.).

1.8 Quantitative Real-time PCR analysis of mRNA

Total RNA from cells or mouse livers was extracted in Trizol according to the manufacturer's instructions (Invitrogen). The RNA was first reverse-transcribed into cDNA using a PrimScript RT reagent Kit (Takara) and then subjected to qPCR with GoTaq qPCR Master Mix (Promega). Real-time PCR was performed on an Applied Biosystem 7300 real-time PCR system, and data were analyzed using the 7300 system SDS software (ABI). The primer sequences (5'～3') were as follows:

HBV pregenomic RNA forward, 5′ AGGAGGC-TGTAGGCATAAATTGG 3′; HBV pregenomic RNA reverse, 5′ CAGCTTGGAGGCTTGAACAGT 3′; β-actin forward, 5′ GAACCCTAAGGCCAACCGT-GAA 3′; β-actin reverse, 5′ CTCAGTAACAGTCC-GCCTAGAA 3′.

1.9 Statistical analysis

Data are expressed as $\bar{x} \pm s$. The results were based on three independent experiments *in vitro* or six animals per group *in vivo*. Comparison between groups was performed by an independent-samples *t*-test. The level of significance was set to $P < 0.05$. Statistical calculations were performed using Prism 5.0 software (Graphpad Software, San Diego, CA).

2 Results

2.1 Inhibition of HBV antigens and replication in cell culture by asRNA

We designed and synthesized three asRNA oligonucleotides targeted to HBV viral mRNAs (Figure 1a) and a randomized oligonucleotide as a control. To increase their stability, the asRNA oligonucleotides were chemically modified by 2'-O-methylation and a butanol tag at their 3'-end.

First, HepG2 cells were co-transfected with pHBV1.3 and the three modified asRNAs or the randomized asRNA as a control. Compared to the group treated with randomized asRNA, the secreted HBsAg in cell culture was reduced by 21% in the P-2987 group, 40% in the X-60 group, and 44% in the X-519 group ($P < 0.05$, Figure 1b). Similarly, the secreted HBeAg was reduced by 32% in the P-2987 group, 51% in the X-60 group, and 54% in the X-519 group ($P < 0.05$, Figure 1c). The pregenomic RNA was reduced by 72% in the X-60 group and 83% in the X-519 group ($P < 0.05$, Figure 1d).

In a similar experiment, asRNAs were transfected into HepG2.2.15 cells. Compared to the group treated with the randomized control asRNA, the secreted HBsAg in cell culture was reduced by 15% in the P-2987 group, 20% in the X-60 group, and 46% in the X-519 group ($P < 0.05$, Figure 2a), and the secreted HBeAg was reduced by 26% in the X-519 group ($P < 0.05$, Figure 2b). The pregenomic RNA was reduced by 58% in the P-2987 group, 85% in the X-60 group, and 70% in the X-519 group ($P < 0.05$, Figure 2c). The HBV DNA copy number in the medium was reduced by 12% in the P-2987 group, 20% in the X-60 group, and 44% in the X-519 group ($P < 0.05$, Figure 2d). In summary, the three asRNAs, X-519, X-60 and P-2987, significantly inhibited HBV antigens expression and HBV replication *in vitro*.

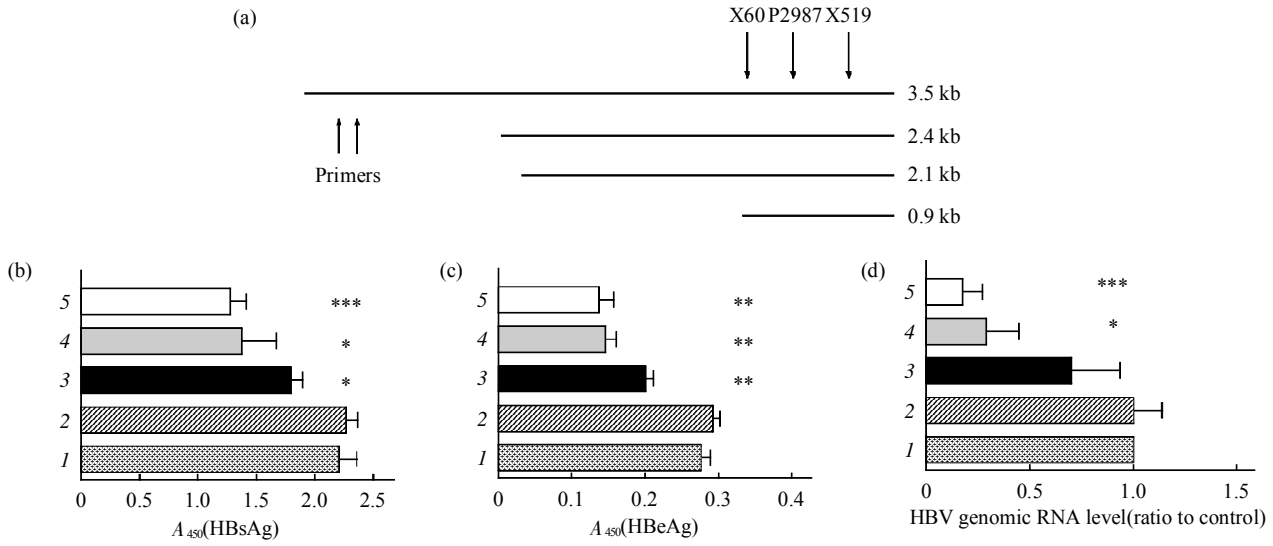

Fig. 1 Inhibition of HBV in the HepG2 cell line co-transfected with pHBV1.3 and asRNA oligonucleotides

(a) Location of target sites of asRNAs. Downward arrows indicated the location of asRNA target sites within the four HBV transcripts. The 3.5-kb transcript is the pregenomic RNA that serve as the template for HBV vrial DNA replication. Upward arrows indicated the target sites of primers for qPCR of pregenomic RNA. (b) HBsAg levels in cell culture post-cotransfection of pHBV1.3 and asRNAs. (c) HBeAg levels. (d) qRT-PCR analysis of HBV pregenomic RNA. *1* (bottom): Control, only pHBV1.3 was transfected; *2*: Random, pHBV1.3 co-transfected with randomized asRNA; *3*～*5*: pHBV1.3 co-transfected with P-2987, X-60, and X-519 respectively. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$ compared to the randomized asRNA group (Student's t-test, $n = 3$).

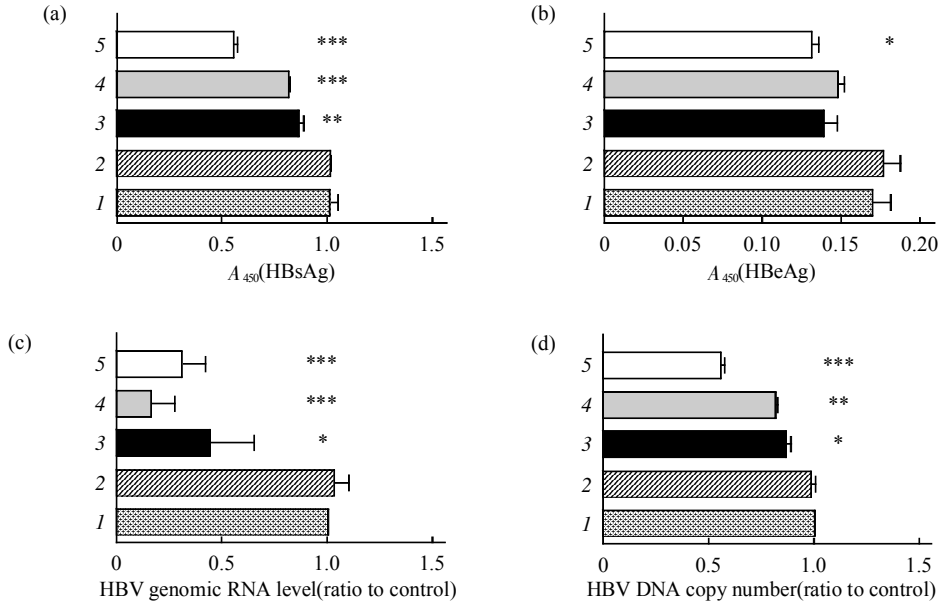

Fig. 2 Inhibition of HBV replication and antigen expression in HepG2.2.15 cells by asRNA oligonucleotides

(a) HBsAg levels in cell culture. (b) HBeAg levels. (c) qRT-PCR analysis of HBV pregenomic RNA expression. (d) qRT-PCR analysis of HBV DNA in cell culture. *1*: Control, no treatment; *2*: Random, treatment with randomized asRNA; *3*～*5*: Treatment with P-2987, X-60, and X-519 respectively. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$ compared to the randomized asRNA group (Student's t-test, $n = 3$).

2.2 asRNA oligos-mediated inhibition of HBV replication in HBV transgenic mice

The ratios of HBsAg and HBeAg levels in the sera after and before treatment were measured by ELISA. There was no significant differences between the three experimental groups and the randomized control group (Figure 3a, b).

Real-time fluorescence qPCR was performed with

total serum DNA to detect HBV DNA copy numbers. We found that there were no significant differences between the three experimental groups and the control group (Figure 3c).

Quantitative Real-time PCR was performed to detect correlative levels of 3.5-kb HBV transcript (pregenomic RNA) with the endogenous control β-actin gene. The pregenomic RNA is a template for reverse transcription for HBV replication. The asRNA X-519 induced a huge decrease 89 % of HBV pregenomic RNA in livers ($P < 0.05$, Figure 3d). These results confirmed the inhibition of HBV replication *in vitro* (Figure 1, 2).

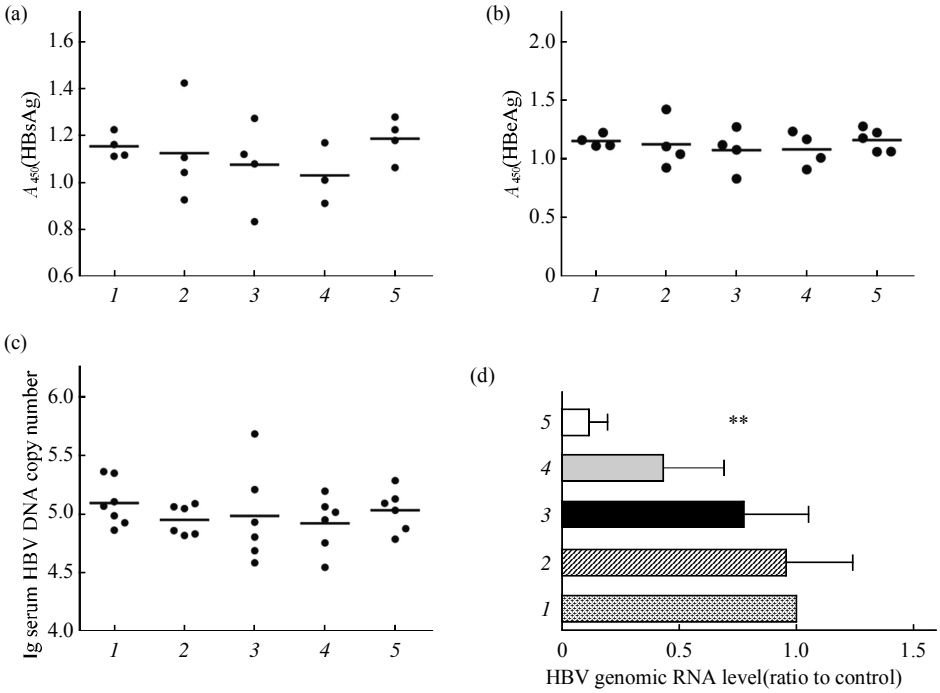

Fig. 3 Inhibition of HBV in HBV transgenic mice treated with asRNA oligonucleotides

The results are based on three independent experiments. (a) HBsAg levels in sera. (b) HBeAg levels. (c) qRT-PCR analysis of HBV DNA copy number in sera. (d) qRT-PCR analysis of HBV pregenomic RNA in liver. *1*: Control, treatment with 0.9% NaCl; *2*: Random, treatment with the randomized asRNA; *3*～*5*: Treatment with P-2987, X-60, and X-519 respectively. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$ compared to the randomized asRNA group (Student's *t*-test, $n = 6$).

2.3 Cationic liposome-X-519 complex-induced inhibition of HBV *in vivo*

Although HBV pregenomic RNA in the liver decreased significantly after X-519 treatment, the HBV antigens (HBsAg and HBeAg) and HBV DNA copy number in sera in HBV transgenetic mice were still at a high level after treatment. To test whether we could enhance the inhibitory effect on HBV in HBV transgenetic mice, asRNA oligonucleotide X-519 was mixed with cationic liposome DMRIE-C and intravenously injected into HBV transgenic mice. The size distribution, PDI, and zeta-potential of liposome-RNA complexes were measured using a zetasizer. The particle diameter was approximately 300 ～400 nm (Table 1). The zeta-potential of DMRIE-C was positive (51.3 mV) before adding asRNA. While more asRNA was added, the negative zeta-potential decreased as expected. After reaching a ratio of 4∶1 (asRNA∶liposome), the zeta potential decreased to a constant (–76 mV), so we predicted that the asRNA adsorbed on the surface of liposome reached saturation (Table 1). We also tested liposome-RNA stability under different conditions,

Table 1 Parameters of liposome-RNA complex under different ratios of RNA oligo to liposome

RNA oligo∶liposome	Zeta potential/mV	Particle size	PDI
1∶1	–69.2	334.5	0.19
2∶1	–71.5	319.3	0.229
4∶1	–76.8	329.1	0.232
8∶1	–76.5	316.4	0.211
DMRIE-C only	51.3	401.3	0.259

such as different ratios of RNA to liposome, as well as different incubation temperatures and times, as shown in Tables 1 and 2. To determine the storage conditions and stability of liposome-RNA complexes before delivery into mice, the samples were incubated at room temperature, 4 ℃ , or 37 ℃ (to mimic mouse body temperature) for 12 h or longer. The particle size and PDI displayed no differences between these groups, indicating that the cationic liposome encapsulation of RNA was stable.

Table 2 Parameters of liposome-RNA complex under different incubation temperatures and times

	RNA oligo：liposome	Particle size	PDI
DMRIE-C	2：1	382.1	0.172
fresh	1：1	313.7	0.19
25℃, 24 h	2：1	332.2	0.138
	1：1	371.3	0.235
37℃, 24 h	2：1	321.6	0.154
	1：1	330.1	0.14
37℃,12 h	2：1	326.2	0.134
	1：1	331.9	0.193
4℃, one week	2：1	305	0.21
	1：1	324	0.186

After the 2-week treatment, there were still no significant changes of HBsAg or HBV DNA copy number in the sera (Figure 4a, b). However HBV pregenomic RNA in the liver was significantly reduced, and cationic liposome encapsulation enhanced the repression from 81% to 91% ($P < 0.05$, Figure 4c).

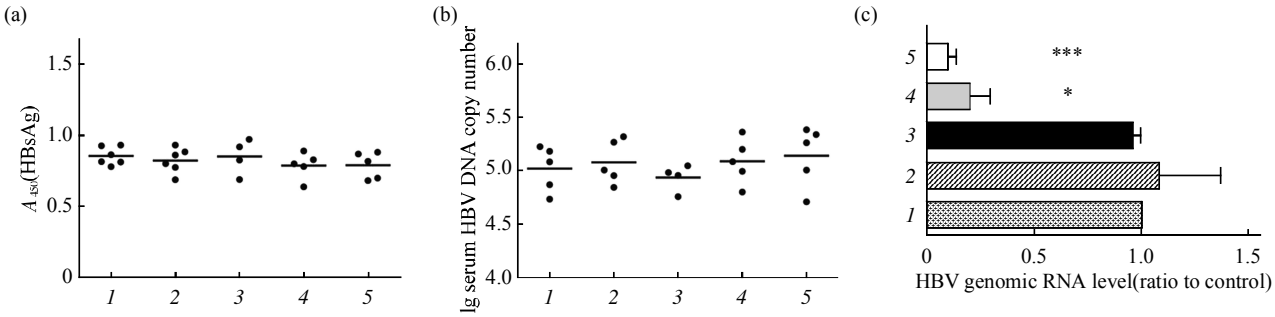

Fig. 4 Inhibition of HBV in HBV transgenic mice treated with cationic liposome-RNA complexes

(a) HBsAg levels in sera. (b) qRT-PCR analysis of HBV DNA copy number in sera. (c) qRT-PCR analysis of HBV pregenomic RNA in liver. *1*: Control, treatment with 0.9% NaCl; *2*: Random, treatment with the randomized asRNA; *3*: ra+lipo, treatment with the randomized asRNA and cationic liposome mixture; *4*: X-519, treatment with X-519; *5*: X-519+lipo, treatment with X-519 and cationic liposome mixture. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$ compared to the randomized asRNA group (Student's t-test, $n = 6$).

2.4 X-519-mediated inhibition of HBV in hydrodynamically injected mice

Secreted HBeAg in mouse serum was monitored from day 0 to day 10 after pHBV1.3 injection, and the data showed that HBeAg reached a very high level the day after injection. By day 10, however, only a low amount of HBeAg could be detected (Figure 5a) when HBsAg was still at a high level. Consistent with the acute circulatory volume overload required for hydrodynamic transfection, sALT activity, like serum HBeAg level, increased sharply on day 1 and returned to baseline levels (< 50 U/L) by day 7 post-transfection (Figure 5b). Thus, we created a mouse model of acute HBV infection in which HBV replication and expression lasts for at least 2 weeks, and we monitored the mice treated with asRNA for 1 week post-hydrodynamic injection.

asRNA oligonucleotide X-519, which had the most efficient inhibitory effect on HBV in the HBV transgenic mice experiments, was delivered everyday *via* the tail vein from the day of pHBV1.3 injection for 1 week. On day 7, mice were bled, and the sera were prepared to measure HBsAg, HBeAg, and HBV DNA copy number; liver samples were collected for HBV pregenomic RNA tests. Compared to the randomized asRNA group, serum HBsAg was reduced by 46% in mouse serum from the X-519 group($P < 0.05$, Figure 5c), and HBeAg was reduced by 51%($P < 0.05$, Figure 5d). HBV pregenomic RNA in the liver was reduced by 60% ($P < 0.05$, Figure 5d), and HBV DNA copy number was reduced by 90% ($P < 0.05$, Figure 5e). In summary, the asRNA oligonucleotide X-519 significantly reduced HBV replication and expression in mice with acute HBV infection.

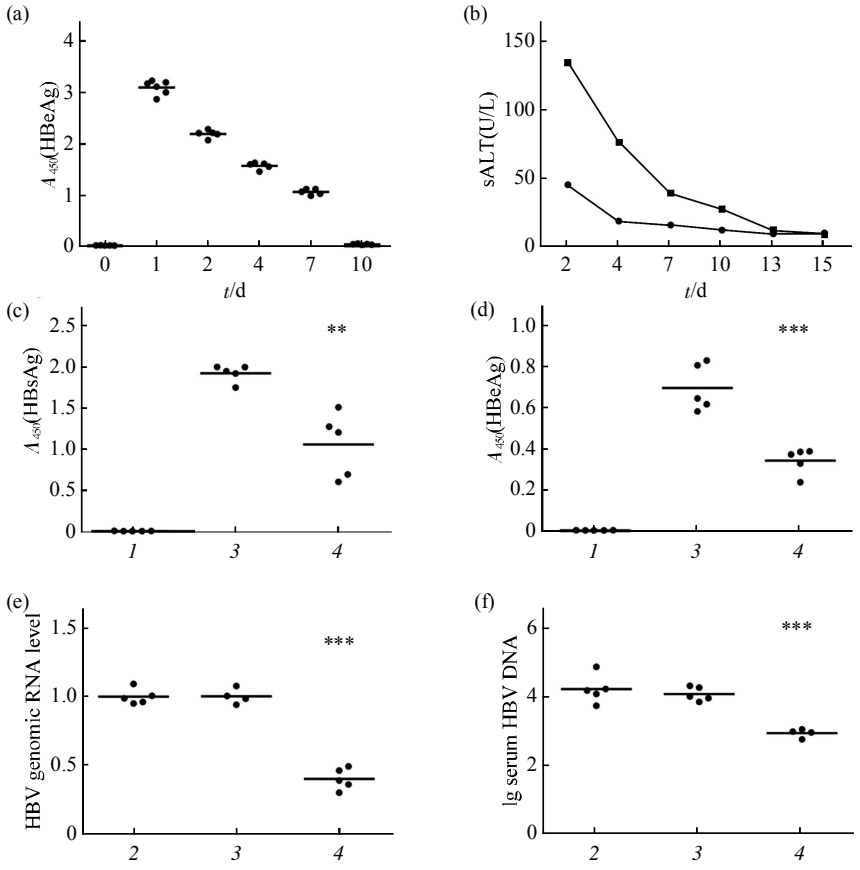

Fig. 5 Inhibition of HBV replication and expression in the hydrodynamic injection mouse model

(a) Changes in HBeAg levels in sera post-hydrodynamic injection of pHBV1.3 from day 0 to day 10. (b) Comparing the sALT levels in the sera post-hydrodynamic injection of pHBV1.3 with no injection from day 0 to day 10. Treatments were performed with asRNAs post-hydrodynamic injection. ●—●: No inject; ■—■: pHBV1.3. (c) HBsAg levels in sera. (d) HBeAg levels. (e) qRT-PCR analysis of HBV pregenomic RNA in liver. (f) qRT-PCR analysis of HBV DNA copy number in sera. *1*: Control, hydrodynamic injection of 0.9% NaCl; *2*: HBV1.3, hydrodynamic injection of pHBV1.3; *3*: HBV1.3+ra, treatment with the randomized asRNA post-hydrodynamic injection of pHBV1.3; *4*: HBV1.3+X-519, treatment with X-519 post-hydrodynamic injection of pHBV1.3. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$ compared to the randomized asRNA group (Student's *t*-test, n=6).

3 Discussion

Currently, the development of an effective anti-HBV therapy is still a challenge. During HBV replication, the HBV genome rapidly accumulates mutations due to the high rate of replication and the lack of proofreading by reverse transcription[1–2]. Thus, the current anti-HBV drugs, including recombinant interferon and nucleotides analogs, such as lamivudine, do not directly affect the HBV pregenomic RNA, and drug resistant mutations are frequent[4, 14–16]. asRNA is a single-stranded RNA that is complementary to an mRNA. When mRNA forms a duplex with a complementary asRNA sequence, translation is blocked. This may occur because the ribosome cannot gain access to the nucleotides in the mRNA or because duplex RNA is quickly degraded by ribonucleases in the cell [5, 17–18]. For specific gene slicing, target gene selection has been one of the most critical considerations [6]. The design for antisense nucleotides against HBV has been focused on viral gene targets that are most conserved.

We designed, synthesized, and modified three asRNA oligonucleotides targeting viral mRNAs and investigated their repressive effects on HBV. The four viral mRNAs of HBV overlap at the 3'-end. Thus, asRNA X-519, which targets the 3'-end of the HBV X protein mRNA, also binds to the other three viral mRNAs, especially the pregenomic RNA related to viral replication (Figure 1a). Our asRNA nucleotides are 20-bp single-stranded RNAs, which are easy to deliver *in vivo*. There is no need to construct vectors[14, 19–24]

that express short hairpin RNAs, which is commonly used for RNA interference *in vivo* and gene therapy. 2'-O-methyl RNAs, which carry a methyl group at the 2'-OH residue of the ribose molecule, display the same behavior as DNA but are protected against nuclease degradation. We also modified the asRNAs with a 3'-end butanol tag that enhanced the stability of the asRNA[6, 12].

Here, we used two cell lines for *in vitro* analysis and two mouse models for *in vivo* experiments. In pHBV1.3-transfected HepG2 cells and HepG2.2.15 cells, HBV were significantly repressed by the modified asRNAs on HBV pregenomic RNA level, antigens expression and HBV DNA copy number. Data from HBV transgenic mice indicates that injection through the tail vein permits delivery of asRNA, as HBV pregenomic RNA levels in the liver decreased by 89% after treatment compared with the randomized asRNA-treated group. Recent research presents the delivery of siRNA or siRNA-producing plasmids against HBV, using anti-HBsAg fusion proteins (single-chain antibody) [20]. Here, the cationic liposome DMRIE-C was chosen as an RNA delivery tool to improve transfection efficiency[25–27]. DMRIE-C is suitable for the transfection of DNA and RNA into eukaryotic cells and also for *in vivo* delivery of DNA. The parameters of the DMRIE-C-RNA complexes indicated that the ratio of 4∶1 (RNA∶liposome) represents saturation of RNA adsorption by the liposome (Table 1 and 2), and the results of animal experiments demonstrated that cationic liposome highly increased the repressive effect of asRNAs on HBV replication in the live, from 81% to 91%(Figure 4).

We also found that the HBV transgenic mouse model may not be suitable for our investigations. Because the HBV genome is integrated into the mouse genome, all of the cell types and tissues carry HBV. Although qRT-PCR indicated that X-519 is efficiently transported into hepatocytes *in vivo* and that HBV replication is repressed in the liver, no changes were detected in the levels of sera antigens and HBV DNA copy number. Thus, we tested an acute HBV infection mouse model by hydrodynamic injection [13, 24]. Hydrodynamic injection of pHBV1.3 dissolved in a high-load volume in a short time is an efficient method to transfect hepatocytes *in vivo*. Different from HBV transgenic mice that contain an integrated HBV genome, pHBV1.3 is mostly transfected into hepatocytes. Overload of liquid injected through the tail vein in a short time causes high pressure and liver injury, which makes the delivery of the HBV genome to hepatocytes more efficient. In this model, the liver is the main tissue carrying the HBV genome and expressing HBV antigens. X-519 efficiently repressed HBV pregenomic RNA, viral antigens and HBV DNA in sera. Thus, our results indicated the potential value of asRNA oligonucleotide X-519 for gene therapy against HBV infection, and cationic liposome may be a useful tool for its delivery.

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反义 RNA 寡核苷酸及其与脂质体的复合物对于乙型肝炎病毒的抑制 *

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摘要 我们设计合成了特异性靶向乙型肝炎病毒(HBV)mRNA 的反义 RNA 寡核苷酸 P-2987、X-60 和 X-519．在瞬时转染 pHBV1.3 质粒(含有 1.3 拷贝的 HBV 基因组)的 HepG2 细胞和整合了 HBV 基因组的 HepG2.2.15 细胞中，转染 2 μmol/L 的反义 RNA 寡核苷酸，ELISA 和实时定量 PCR 结果表明，这 3 条寡核苷酸可以明显抑制 HBV 的复制和抗原表达．在 HBV 转基因鼠中，尾静脉注射反义 RNA 寡核苷酸，结果表明，肝脏中 HBV 的复制得到了抑制，但是血清中抗原含量和 HBV DNA 拷贝数没有明显变化．反义 RNA 寡核苷酸 X-519 与脂质体的复合物可以增强其对于 HBV 在肝脏中复制的抑制作用．在通过高压尾静脉注射 pHBV1.3 质粒建立的 HBV 急性感染模型中，反义 RNA 寡核苷酸 X-519 可以显著地抑制 HBV 在肝脏中的复制以及降低血清中病毒抗原水平和 DNA 拷贝数．上述实验结果说明，X-519 及其与脂质体的复合物对于 HBV 的复制和抗原表达起到明显的抑制作用，可能作为一种潜在的针对 HBV 的基因治疗药物．

关键词 乙型肝炎病毒，反义 RNA 寡核苷酸，HBV 转基因鼠，高压尾静脉注射，反义 RNA 寡核苷酸与脂质体复合物
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