COMMENTARY

Potential clinical application of RNAi-based therapeutic strategies for treatment of chronic hepatitis B

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INTRODUCTION

In a previous publication[1] on potential application of RNAi techniques for treatment of diseases, it was pointed out that infectious diseases can be one of the major fields that the techniques may have great potential for clinical application. Among hundreds of infectious diseases caused by various pathogens that threatening human health, hepatitis B virus (HBV) infection is one of the infectious diseases that has relatively high prevalence, chronicity, larger disease burden, threatening the patients' life after leading to cirrhosis and hepatocellular carcinoma. It is estimated by the World Health Organization (WHO) that the number of people infected with hepatitis B virus [hepatitis B surface antigen (HBsAg) positive] was 257–360 million globally,[2,3] and in 2015, 887 000 deaths in the world were attributable to HBV infection, mostly due to complications of hepatitis B, such as cirrhosis, hepatocellular carcinoma and liver failure. The situation and hazards of hepatitis B may be worse than many people thought about. As high as one-third of the global population are infected with HBV at some point of their lives.[3] Infants and young children are at high risk for acquiring chronic HBV infection, 80%–90% of infants infected during the first year of life and 30%–50% of children infected before the age of 6 years develop chronic HBV infection.[2] Many of the patients with chronic HBV infection are facing severely decreased quality of life and even threat to their lives. Although there are already very effective vaccine to prevent the infection and effective (but not curative) and safe antiviral agents for treatment of the disease, there are still practical problems that compel researchers to study and develop better therapeutic strategies.

CURRENT TREATMENTS OF HEPATITIS B, REASONS FOR DEVELOPMENT OF BETTER AND NEWER THERAPIES

Since introduction of genetically engineered vaccine against hepatitis B virus, the prevalence of hepatitis B infection has been remarkably reduced. For example, the prevalence of HBV or HBsAg carriage rate in China was reduced from 9.7% of 1992–1994 to 7.2% of 2007, which meant that HBV infection was avoided in about 30 million people within a period of about 15 years.[4] This is one of the greatest successes and achievements in disease prevention. However, China was still facing treatment of as many as 20 million patients who already have had chronic HBV infection. On the other hand, vaccination coverage can hardly reach 100%. According to an earlier report,[5] the coverage rate of vaccination against HBV among children under the age of 12 months was 88.5% in urban and 62.7% in rural areas in 1999, although there was great difference among provinces. In African countries, the birth dose of HBV vaccine is introduced only in 11 of 47 countries.[6] The children who were not inoculated with vaccine against HBV may still expose to the risks of infection with HBV in their later life.

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The current antiviral therapies for chronic hepatitis B (CHB) have certain limitations. The major therapeutic approaches to CHB include 1) pegylated interferon (PEG-IFN) and 2) nucleoside/nucleotide [nucleos(t)ide] analogues antiviral agents currently represented by entecavir and tenofovir. PEG-IFN therapy takes a finite period of administration and can achieve a moderate suppression rate of HBV DNA, HBsAg seroconversion and histological improvement; however, it has numerous adverse reactions, including depression, paresthesia, myelosuppression, and flu-like symptoms. The nucleos(t)ide analogues do not result in serious adverse reactions, and the later developed products showed higher rates of virological response, HBeAg (hepatitis B e antigen) and HBsAg seroconversion and histological improvements. Despite these encouraging progresses, the overall therapeutic effects of these two therapies are far from ideal, i.e., they cannot result in high rate of viral clearance, complete histological recovery and good prognosis. Because of the less ideal therapeutic effects, most of the patients with CHB have to take nucleos(t)ide analogues for life.[3,7] This will bring the problems of financial difficulty and reduced compliance to the medications.

In addition to the suboptimal therapeutic effects, in fact there are some other less ideal factors with these therapies. Daily or several times daily administration of medicines for chronic diseases seems to be very common and normal. However, if two different therapies with exactly the same therapeutic effects have entirely different ways of administration, e.g., one is given everyday and the other given once a month or even once every 3 months, the latter must be regarded as highly superior and will be able to minimize the problem of compliance.[8]

On the other hand, theoretically, if an antiviral agent can act on and inhibit a few different stages (steps) of viral replication, it may be able to completely stop and clear the virus from the body of the host.

Taken together, although there are safe and effective vaccine against HBV, and effective therapeutic approaches, there are still urgent needs for developing newer and better therapeutic strategies against chronic HBV infection that not only can result in functional cure of CHB, but also can completely clear the virus and achieve virological and histological cure.[9,10]

OVERVIEW OF RNAI-BASED STRATEGIES FOR TREATMENT OF CHRONIC HEPATITIS B

Earlier studies on RNAi-based therapeutic approaches to treatment of CHB were reported about 10 years ago.[11-13] The targets of the RNAi interference included the genes of hepatitis B core antigen (HBcAg), HBsAg (including Pre-S1, Pre-S2 and S genes) and HBV covalently closed circular DNA (cccDNA) (Table 1). Theoretically, the more the HB genes are silenced, the more effectively and completely the replication of HBV is inhibited, although no reports on direct comparison of the effects in this regard have been found. Studies on such comparisons can be very important, and therefore should be designed and conducted in the near future.

The setting of the studies for testing the effects and safety of the RNAi-based anti-HBV agents were largely cultured cells (HepG2 cells), transgenic mice, and none-human primates (Table 1). All the preclinical studies on RNAi-based anti-HBV agents consistently showed significantly large proportion reduction of HBsAg even after a single dose of the agents.[11-15,19] This is a remarkable difference as compared with either PEG-IFN or nucleos(t)ide analogue therapies.

In one clinical study, the safety, tolerability and pharmacokinetics of RNAi-based anti-HBV agent (ARC-520) were observed in human volunteers in a randomized controlled trial (RCT) (Table 1). In another clinical trial, efficacy of ARC-520 was evaluated.[9] In a phase 2a, randomized, double-blind, placebo-controlled clinical study conducted in HBeAg-negative adult CHB patients, a single intravenous dose of ARC-520 up to 3 mg/kg showed good tolerability, and dose-dependent reduction of HBsAg; serum HBsAg was reduced by up to 50% after a single dose of 2 mg/kg and statistically significant reductions persisted for up to 43–57 days.[9] These preliminary results of the clinical trials may suggest that rigorously designed study on RNAi-based anti-HBV agents may have the potential of virological cure of CHB, and millions of patients with CHB may hopefully achieve complete recovery although some of the studies (preclinical[11-13] and clinical[18]) did not pay sufficient attention to the safety and adverse effects/events of the study agents.

POTENTIAL ADVANTAGES OF WELL DESIGNED RNAI-BASED ANTI-HBV THERAPIES

Nucleos(t)ide analogues may have limited target step of viral replication inhibition of polymerse-reverse transcriptase. But RNAi-based approaches may have many more targets to interfere with, including silencing of mRNAs of the polymerase-reverse transcriptase gene, HBcAg gene, HBsAg gene (including the Pre-S, Pre-S1, Pre-S2 and S genes), HBx protein gene, HBV cccDNA and even the viral gene that has already integrated into the host gene.

The most important advantage of RNAi-based anti-HBV treatments is direct and potent inhibition of viral proteins production, especially reduction of HBsAg by up to 50%–80% or even higher proportions. Such a potent inhibition of viral proteins production would probably lead

TABLE 1 Effects and safety of preclinically and clinically tested RNAi-based anti-hepatitis B virus agents

Name of RNAi- based approach	Target of RNA interference	Setting of testing	Effect on HBV replication	Adverse effects	No of reference, author name and year	Way of delivery
Chemically synthesized siRNA	S, Pre-C and C genes of HBV	Mice transfected with pHBV 1.5 construct; HuH7 and HepG2 cells	Significant reduction of HBsAg (70%) and HBeAg (80%) expression; HBeAg decreased to 4.6–4.9 folds	Not mentioned	11. Klein C, et al. 2003	Direct injection of the siRNAs into the tail vein
Chemically synthesized siRNA4 and 6	S and Pre-C genes	HepG2.2.15 cells	HBsAg 76.2% knock down; HBeAg decreased by 73.8% and 72.8%	No apoptosis of the cells was observed	12. Chen Z, et al. 2005	Transfection with Oligofectamine
HBV-specific 21-bp short hairpin RNAs (shRNAs) and siRNA	HBsAg and HBcAg genes	HepG2.2.15 cells and a mouse model for HBV replication	HBsAg secretion reduced by 80%; HBV DNA by 40%–60% in cell culture, and HBsAg and HBcAg-positive cells in mice liver was reduced by 55.7% or 92.0%	Not mentioned	13. Ying RS, et al. 2007	Cells were transfected with liposome metafectene; the shRNA was dissolved in Ringer's solutions and injected into the tail vein
Altritol-containing synthetic siRNAs (ANA siRNAs)	Core/Pre-S1/2/ S genes and HBx gene	HBV transgenic mouse model	Inhibition of HBV replication by approximately 50%	Non-specific immune stimulation was significantly attenuated; no toxicity was induced	14. Hean J, et al. 2010	Lipoplex
NAG-MLP and chol-siHBV-74, -75, -76, -77	HBV cccDNA	Transgenic mice, NHPs (non-human primates); HBV genotype coverage 99.64%	HBsAg was reduced > 2 log10 (100-fold or ≥99% knockdown) for 1 month, > 1000-fold, and 85% knock down of HBeAg; 81%–96% reductions in HBV markers at day 29	No serious adverse reactions were observed	15. Wooddell CI, et al. 2013	Coinjection with NAG-MLP and chol-siHBV, hepatocyte-tropic and targeted
ARC-520	HBV transcripts	Clinical trial, HBeAg-negative CHB patients, 22 (8,8,6 cases)	HBsAg was reduced by 31% at 1 mg/kg, by 51% at 2 mg/kg. Significant HBsAg reduction persisted 40 days.	Mild or moderate AEs occurred in 4 cases, no SAE was observed	16. Yuen M, et al. 2014	Coinjection of NAG-MLP and chol-siRNA
ARC-520	cccDNA-derived viral mRNA transcripts	Human volunteers, RCT, double blind, placebo-controlled trial, 9 cohorts (6 persons in each)	Not applicable (the study was not designed for efficacy evaluation)	AE rate was the same as placebo, no SAE; 2 cases had suspected hypersensitivity (flushing, urticarial rash)	17. Schluep T, et al. 2016	ARC-520 injection administered intravenously
ARC-520	HBV transcripts	Human phase II clinical study, 8 cases X5 cohorts, 6:2 active/placebo and chimpanzees	HBsAg was reduced by 0.3 log10; 14 log10 reduction in NUC-naive CHB patients. HBV DNA by 4 Log10 in chimpanzees	Not mentioned	18. Wooddell CI, et al. 2017	Single intravenous injection
siRNA-loaded lipid nanoparticles (GalNAc/PEG-LNPs with HBV-siRNA mix)	Not mentioned	Chimeric mice with human liver	Significant reduction of HBV genomic DNAs and their antigens (HBsAg, HBeAg and HBcrAg) by 60%–80%	No any sign of toxicity was observed	19. Sato Y, et al. 2017	Lipid nanoparticles (LNPs) highly specific delivery to hepatocytes

Notes. HBV, denotes hepatitis B virus; siRNA, small interfering RNA; shRNA, short hairpin RNA; HBsAg, hepatitis B surface antigen; HBcAg, hepatitis B core antigen; HBeAg, hepatitis B e antigen; HBcrAg, hepatitis B core-related antigen; CHB, chronic hepatitis B; cccDNA, covalent complimentary circular DNA; AE, adverse event; SAE, serious AE.

to clearance of the virus within a relatively short period of time.

Other advantages of ARC-520 is that it is an RNAi trigger with broad genotype coverage. HBV has 9 major genotypes. An anti-HBV agent should cover all or most of the genotypes that frequently cause human disease. Conserved sequences were identified that could be used to generate RNAi triggers cross-reactive with >90% of known HBV genomes. Preliminary observation also suggests that rigorously designed RNAi-based anti-HBV agent may have the superiority of long *in vivo* half life, therefore, it is possible that the agents may be given at an interval of at least several weeks.

For RNAi-based anti-HBV therapeutic approaches, specifically liver-targeted delivery should be extremely important in addition to certain measures to protect the siRNA or shRNA from degradation by ribonucleases that present in blood stream and tissues. The recently reported studies[15-19] applied different methods to make their RNAi-based anti-HBV agents highly hepatotropic, which is a good paradigm for further studies in this field. Direct comparison between the liver- or hepatocyte-targeted and non-targeted delivery may also be required. Liver cell targeted delivery methods are important for the efficient silencing of HBV genes.

In summary, evidences provided by preclinical and clinical studies on treatment of chronic HBV infection with RNAi-based anti-HBV approaches are very promising, and with more rigorously designed larger scale clinical trials in different ethnic and regional populations that are expected to be conducted and reported, a new era of applying RNAi-based antiviral therapies for viral hepatitis and for many other infectious diseases may soon arrive.

CONFLICT OF INTEREST

I have no conflict of interest to declare with regard to this manuscript.

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