DOI: 10.35772/ghm.2024.01078

Clinical potential of SAG-524: A novel HBV RNA destabilizer with a unique mechanism of action

Yasuhito Tanaka*

Department of Gastroenterology and Hepatology, Faculty of Life Sciences, Kumamoto University, Kumamoto, Japan.

Abstract: SAG-524 is a novel, oral HBV RNA destabilizer developed to address the limitations of treatment with nucleos(t)ide analogues (NAs), which are effective against HBV DNA but show limited efficacy in reducing hepatitis B surface antigen (HBsAg) levels. SAG-524 exerts its effect by destabilizing HBV RNA by shortening the poly(A) tail, which leads to a significant reduction of both pgRNA and PreS/S mRNA. This destabilization seems to be specific for HBV RNA molecules. The mechanism involves the recruitment of PAPD5/7 by ZCCHC14 to the HBV RNA, where guanine is incorporated into the poly(A) tail to protect against degradation. SAG-524 disrupts this process by directly targeting PAPD5, thus destabilizing HBV RNA. In preclinical trials, oral administration of SAG-524 reduced serum HBsAg levels in HBV-infected PXB mice. When combined with NAs or capsid assembly modulators (CAMs), significant reductions in HBsAg, HBV DNA, and intrahepatic covalently closed circular DNA were observed. Safety studies conducted over 13 weeks in mice and monkeys revealed no significant toxicity, demonstrating the drug demonstrated a favorable safety profile. In conclusion, the novel mechanism of action, high oral bioavailability, and strong suppression of HBsAg make SAG-524 a promising candidate for future therapeutic use. The potential for combination therapy with NAs or CAMs underscores its capacity to contribute to achieving a functional cure for chronic HBV infection.

***Keywords*:** HBV, SAG-524, combination therapy

Introduction

Currently, nucleos(t)ide analogues (NAs) and pegylated interferon are used to treat chronic hepatitis caused by persistent hepatitis B virus (HBV) infection. However, NAs, the most commonly used treatment, do not significantly reduce serum hepatitis B surface antigen (HBsAg) levels, which limits their potential for achieving a functional cure (HBsAg loss). Despite the antiviral efficacy of NAs in controlling HBV replication, their ability to clear HBsAg remains minimal, with low seroclearance rates observed over extended treatment periods. As a result, the likelihood of achieving a functional cure with current therapies is considered low. Therefore, there is an ongoing effort to develop new therapeutic agents aimed at achieving a functional cure for HBV infection (*1*).

One class of direct-acting antiviral agents, HBV RNA inhibitors, has shown promising results in suppressing viral protein expression and genome replication. This article outlines the possible mechanisms of action of HBV RNA inhibitors and highlights new therapeutic agents currently under development and in clinical trials.

What are HBV RNA inhibitors?

The HBV genome exists as a covalently closed circular DNA (cccDNA) in the nuclei of hepatocytes. Several mRNAs are transcribed from the cccDNA, including a 3.5 kb mRNA that functions as pregenomic RNA, and 2.4 kb and 2.1 kb mRNAs encoding the various forms of HBsAg. RNA inhibitors target these RNAs and include small interfering RNAs (siRNAs) and antisense oligonucleotides (ASOs); examples of both are currently undergoing clinical trials. Additionally, a new class of drugs, known as RNA-binding protein inhibitors, is under development. These agents aim to suppress HBsAg production and contribute to the restoration of the host immune response, thereby achieving a functional cure (Figure 1).

Antisense oligonucleotides

ASOs are short, single-stranded DNA or RNA sequences, typically fewer than 20 nucleotides in length,

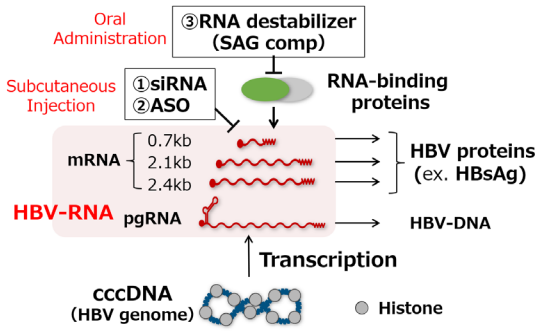

Figure 1. Targeting HBV replication: RNA inhibitors as a strategy for suppressing HBs antigen production. The HBV genome exists as cccDNA in hepatocyte nuclei, where it transcribes various RNAs for viral replication. RNA inhibitors like ①siRNA, ②antisense oligonucleotides (ASO), and our newly developed ③RNA-binding protein inhibitors target HBV-RNA to block its replication. These inhibitors can effectively suppress HBs antigen production.

that are complementary to a specific target sequence. By binding to RNA molecules in a complementary manner, ASOs regulate their function. Antisense DNA binds to complementary RNA and the RNA component of the DNA/RNA hybrid is quickly degraded by ribonuclease H (RNaseH1). ASOs can be designed with various combinations of DNA and modified nucleic acids to target entire RNAs or act on specific mRNA precursors or microRNAs (miRNAs), resulting in diverse mechanisms of action (*2,3*). Furthermore, ASOs can be modified to target functional sites on RNA, allowing them to exert a range of effects, such as inhibiting or enhancing RNA function. This versatility suggests the potential for their development as drugs tailored to specific disease mechanisms. ASOs designed for anti-HBV therapy specifically target the degradation of viral RNA to block the expression of viral proteins. With modified DNA strands that resist degradation by nucleases, ASOs bind to HBV RNA transcribed from cccDNA, in the nucleus and cytoplasm, and enable its cleavage by RNaseH1, thereby inhibiting viral protein translation and generating antiviral activity. Notably, these ASOs inhibit not only pregenomic RNA (pgRNA) but also the synthesis of HBsAg, which cannot be targeted by nucleoside analogues. Therefore, they are promising agents that may reduce HBsAg levels and potentially induce a functional cure for HBV infection.

A phase III trial of GSK3228836 (bepirovirsen), which lacks asialoglycoprotein modification, is currently ongoing. In this trial, subcutaneous injections are administered initially twice weekly, then once per week from the third week onwards (*4*). This treatment regimen results in a significant reduction of serum HBsAg. In particular, the HBsAg clearance rate (< 0.05 IU/mL) in the 300 mg dose group was 9% in the subjects receiving NAs and 10% in the subjects without combination therapy. Notably, among patients with baseline HBsAg levels below 1,000 IU/mL, a functional cure was achieved in 16% of the NA combination group and 25% of the non-combination group (*5*). Interestingly, in the cases where the HBsAg levels decreased, an elevation in ALT levels was observed during the course of treatment, suggesting the potential induction of HBV-specific immune responses.

RNA-binding protein inhibitors (HBV RNA destabilizers)

A new class of drugs known as RNA-binding protein inhibitors has been developed. RG-7834, a novel, oral HBV agent, belongs to the dihydroquinolizinone (DHQ) class and functions by inhibiting PAPD5/7, RNA-binding proteins that stabilize HBV RNA. By inhibiting these proteins, RG-7834 acts as an RNA destabilizer, promoting the degradation of HBV RNA (*6*).

Recently, from a library of approximately 30,000 compounds, we identified a new hit compound, SAG-524, with strong anti-HBV activity (*7*). This report outlines the developmental status of SAG-524. First, we analyzed the mechanism of action using the BRIC assay, a method for measuring intracellular RNA degradation rates. By incorporating bromouridine during RNA synthesis and conducting a time-chase experiment, we found that SAG treatment specifically degraded HBV RNA without significantly affecting GAPDH or albumin RNA, showing that SAG selectively degrades HBV RNA. Further analysis revealed that treatment with SAG significantly reduced the intracellular levels of pgRNA and PreS/S mRNA, leading to a shortening of the poly(A) tail on HBV RNA, which suggests destabilization of the RNA. PAPD5 is known as a poly(A) polymerase that stabilizes mRNA by incorporating random guanines, which confer resistance to deadenylation, and recent reports have identified PAPD5 as a key factor in HBV RNA stabilization. We also investigated the interaction between SAG compounds and PAPD5. In thermal shift assays, we observed that PAPD5 was degraded at 52°C but, when treated with SAG, it remained stable at higher temperatures, indicating that SAG interacts with PAPD5. However, there was little interaction with PAPD7. Additionally, in HepG2.2.15 cells, SAG treatment reduced HBsAg concentrations in the culture supernatant, but this effect was negated when siPAPD5 was introduced. These results suggest that SAG compounds specifically and significantly destabilize HBV RNA through interaction with PAPD5 (Figure 2).

Next, we investigated combination therapy of SAG-524 with a NA (Entecavir, ETV), which is expected to be used in clinical settings, using HBV-infected chimeric mice. While ETV monotherapy significantly reduced HBV DNA levels, it did not result in a noticeable reduction in HBsAg. However, the addition of orally administered SAG-524 led to a marked reduction in both serum HBsAg and HBV DNA levels,

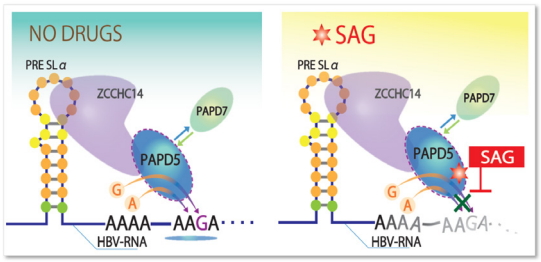

Figure 2. Summary of the mechanism of action of SAG compounds. The HBV RNA stem-loop recruits ZCCHC14 as a scaffold to form a complex with PAPD5. SAG-525 reduces HBsAg levels by acting through PAPD5.

and also decreased intrahepatic cccDNA. Additionally, we evaluated the safety of the drug in animal models. Cynomolgus monkeys, a large animal species, were administered a high dose of 1000 mg/kg of SAG-524 daily for 13 consecutive weeks. No significant abnormalities were observed. Furthermore, pathological examination of the liver, kidneys, brain, nervous system, and other tissues in high-dose treated monkeys revealed no signs of toxicological abnormalities.

In summary: *i*) Combination therapy of orally administrable SAG-524, which destabilizes HBV RNA and strongly suppresses HBsAg, together with a nucleos(t)ide analogue, reduced HBsAg, HBcrAg, and HBV DNA in serum, and intrahepatic cccDNA, in HBV-infected chimeric mice. This suggests the potential for achieving a functional cure; *ii*) Safety tests using large animal models showed no significant toxicity, demonstrating good tolerability. Clinical application is now being pursued.

Conclusion

Prolonged exposure to high levels of viral antigens may lead to exhaustion of the host's immune response, contributing to persistence of HBV infection. Therefore, HBV RNA inhibitors, which aim to reduce HBsAg levels, are considered as an effective therapeutic strategy for controlling HBV infection (*8*). However, some investigators have reported HBsAg rebound after the cessation of RNA inhibitor therapy. In clinical practice, careful attention must be given to the sustainability of the therapeutic response. To enhance the durability of treatment effects, combination therapies that utilize other mechanisms, such as immunotherapy, may be necessary to efficiently achieve a functional cure. The addition of immune-enhancing treatments could provide further support by reactivating the immune system and preventing viral rebound, which is crucial for the long-term control of HBV.

Funding: This work was supported by a grant-in-aid from the Research Program on Hepatitis from the Japan Agency for Medical Research and Development (AMED JP24fk0310518).

Conflict of Interest: Yasuhito Tanaka received Honoraria from AbbVie GK, Gilead Sciences, Inc, Chugai Pharmaceutical Co., Ltd. and GlaxoSmithKline PLC, and Research funds from AbbVie GK, FUJIREBIO Inc, Sysmex Corp, Janssen Pharmaceutical K.K., GlaxoSmithKline PLC, and Gilead Sciences, Inc.

References

1. Chang ML, Liaw YF. Emerging therapies for chronic hepatitis B and the potential for a functional cure. Drugs. 2023; 83:367-388.
2. Crooke ST, Baker BF, Crooke RM, Liang XH. Antisense technology: An overview and prospectus. Nat Rev Drug Discov. 2021; 20:427-453.
3. Roberts TC, Langer R, Wood MJA. Advances in oligonucleotide drug delivery. Nat Rev Drug Discov. 2020; 19:673-694.
4. Yuen MF, Heo J, Jang JW, *et al.* Safety, tolerability and antiviral activity of the antisense oligonucleotide bepirovirsen in patients with chronic hepatitis B: A phase 2 randomized controlled trial. Nat Med. 2021; 27:1725-1734.
5. Yuen MF, Lim SG, Plesniak R, *et al.* Efficacy and safety of bepirovirsen in chronic hepatitis B infection. N Engl J Med. 2022; 387:1957-1968.
6. Mueller H, Wildum S, Luangsay S, *et al.* A novel orally available small molecule that inhibits hepatitis B virus expression. J Hepatol. 2018; 68:412-420.
7. Watanabe T, Hayashi S, Zhaoyu Y, Inada H, Nagaoka K, Tateyama M, Tanaka Y. A novel, small anti-HBV compound reduces HBsAg and HBV-DNA by destabilizing HBV-RNA. J Gastroenterol. 2024; 59:315-328.
8. Ko C, Michler T, Protzer U. Novel viral and host targets to cure hepatitis B. Curr Opin Virol. 2017; 24:38-45.

Received October 18, 2024; Revised December 16, 2024; Accepted December 25, 2024.

Released online in J-STAGE as advance publication December 27, 2024.

**Address correspondence to*:
Yasuhito Tanaka, Department of Gastroenterology and Hepatology, Faculty of Life Sciences, Kumamoto University, Honjo 1-1-1, Chuo, Kumamoto 860-8556, Japan.
E-mail: ytanaka@kumamoto-u.ac.jp