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Antiviral Agents in Hepatitis B virus Systematic Review Structural Analysis of Drug Resistance Mechanisms

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Received Date: April 12, 2023 | Accepted Date: April 22, 2023 | Published Date: April 30, 2023

Citation: Krishnasarmapathy, Saihiti Sarma Pathy, (2023), Antiviral Agents in Hepatitis B Virus Systematic Review Structural Analysis of Drug Resistance Mechanisms, *Clinical Trials and Case Studies*, 2(1); **DOI:**10.31579/2835-835X/022

Abstract

Tenofovir (TFV) is a widely used treatment for chronic hepatitis B virus (HBV) infection. There is a high genetic barrier to the selection of TFV resistance-associated mutations (RAMs), but the distribution and clinical significance of TFV RAMs are not well understood We carried out a systematic literature search. since the first FDA approval of Lamivudine in 1998, many nucleo(t)side analogs such as Lamivudine, Adefovir, and Entecavir have been used. However, they only inhibit DNA synthesis, and if their administration is stopped a viral breakthrough can develop, making long-term administration necessary, ultimately followed by the development of resistance. Tenofovir has been developed and drug-resistant mutations have decreased significantly, but the problem of resistance due to long-term drug use still remains, along with the drug safety problem. In this review, we introduce the recent trend in the development of hepatitis B treatment agents treatment for hepatitis B (drug repositioning) without resistance and which target the various life cycles of HBV.

Keywords: hepatitis B virus; anti-HBV agents; drug resistance

Introduction

Over 300 million people, about 4% of the world's population, are chronically infected with hepatitis B virus (HBV), and a significant number of these patients also suffer from liver diseases such as cirrhosis and liver cancer. In East Asia (especially Taiwan, Japan, Korea and China), HBV is prevalent, with the number of infected people in Korea estimated to be over 2 million, accounting for 60~70% of chronic liver diseases (1, 2). In over 60% of the cases in Korea the infection route for chronic hepatitis B is vertical transmission from mother to infant at childbirth. Treatments for hepatitis B are interferon alpha injections including Peg-interferon and orally-administered nucleo(t)side analogs. Interferon agents have a fixed administration period and are expected to improve the biochemical and histological findings due to their virus-suppressing effect and immune- modulating action, but their use has declined due to the low treatment reaction and adverse side effects. As for nucleo(t)side analogs, they only inhibit DNA synthesis, and if their administration is stopped a viral breakthrough can develop, making long-term administration necessary, ultimately followed by the develop- ment of resistance. Recently, Tenofovir (TDF) has been developed and drug-resistantmutations have decreased significantly, but the problem of resistance by long-term drug use still remains, along with the drug safety problem, due to the treatment characteristics of chronic hepatitis B (3). As such, this paper examines the recent trend in the development of hepatitis B treatment agents. The preparation of Tenofovir disoproxil fumarate is (R)-(1-(6-amino-7H-puri-7-yl) propan-2-yloxy)methyl phosphoric acid undergoes condensation with Chloro methyl isopropyl carbonate in the presence of Nmethyl pyyrolidine as a solvent to give (R)-(((1-(6-amino 9H-purin-9-yl)propan2 yloxy)methyl)phosphoryl)bis(oxy)bis(methylene) isopropyl dicarbonate followed by saltification reaction with fumaric acid in IPA as a solvent media to give Tenofovir disoproxil fumarate

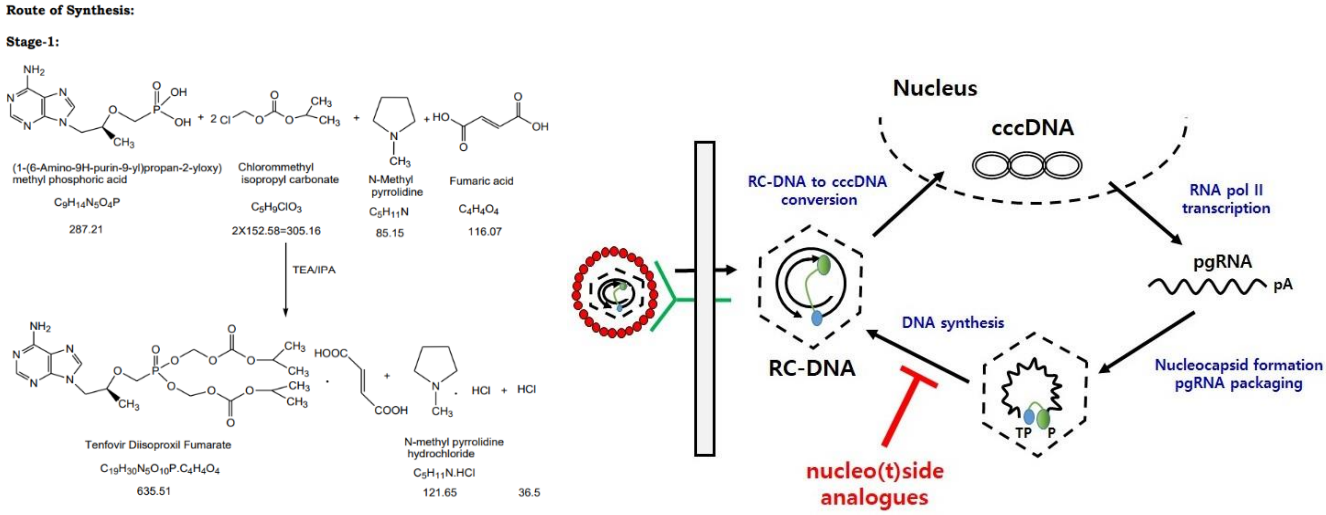


Figure 1: Nucleo(t)side analogues for inhibition of HBV replication.

Characteristics and life cycle of HBV

HBV is a 3.2kbp partially double-strand DNA virus which exists as a complete form of Dane particles with infectivity surrounded by capsid and envelop. The DNA is enclosed by a capsid made of core proteins, which are in turn enclosed by surface proteins. HBV characteristically infects only liver cells (hepatotropic) and causes persistent infection without the degeneration of infected cells (non-cytopathic)[1]. When the HBV virion infects the liver cells, surface proteins are released. Then the viral gene inside the capsid enters into the nucleus and the partially double-stranded HBV DNA transforms into acircular type of cccDNA. The viral RNA produced from the cccDNA produces core and surface proteins and polymerase, and encapsidation progresses in the cytoplasm with pre-genomic RNA (pgRNA), which can be converted to the original viral DNA. After the conversion from pgRNA to DNA, the HBV virion goes through the budding process, after which it can infect or re-infect the surrounding liver cells, causing persistent proliferation (4). pgRNA is transcribed to the DNA within the capsid, and at this time nucleoside analogs cut in to the newly synthesized DNA strand anterminate the synthesis. HBV polymerase consists of four domains, and various drugs have been developed to target the reverse transcriptase (RT) domain where DNA is synthesized from RNA [2-4] and the structural changes of the active site due to mutations of the RT domain are the cause of drug resistance (Fig. 1).

Discussion

Summary of key findings

TFV is a safe and effective treatment choice for CHB in themajority of cases, and large case series have not raised significant concerns about clinically significant drug resistance. However, it is important to consider the potential for the emergence of

resistance, demonstrated by persistent viraemia on therapy and/or reduced virologic suppression in vitro. Based on existing evidence, TFV resistance seems likely to depend on the selec-tion of suites of mutations (most commonly including L180M, A181V/T, M204I/V and/or N236T), overlapping with RAMs that allow escape from other NA drugs. There is also a suggestionthat, rarely, single mutations can confer TFV resistance, best demonstrated for S78T [5-6].

Notably, the literature to date is limited and heterogenous,and there remains a lack of evidence about the frequency andlikely impact of proposed TFV RAMs, either within individual patients or at population level. At present, we have tackled this uncertainty by dividing our catalogue of polymorphisms into a ‘long-list’ (all reported RAMs) and a ‘short-list’ (RAMswith the best evidence-base of support).

Tools that have been designed to identify drug resistance maybias against detection of relevant mutations if they do not scrutinise all relevant sites that contribute to reducing TFV susceptibility. For example, ‘TRUGENE’ a commercially available HBV drug resistance interpretation system, captures

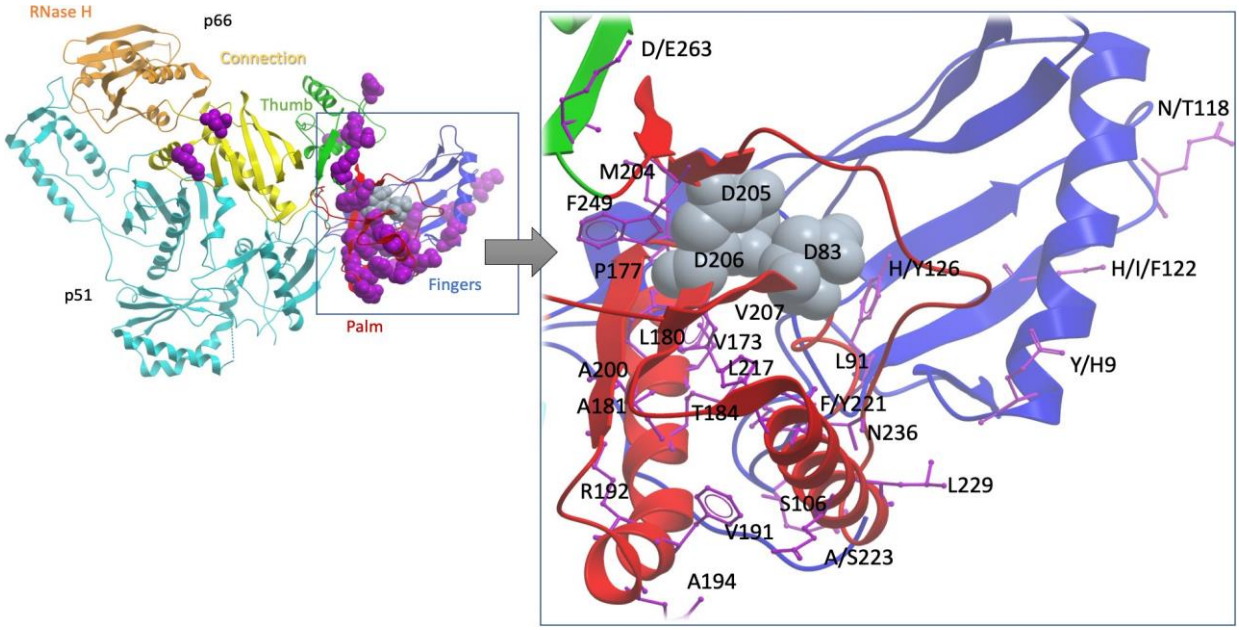


Figure 2: Cartoon to show the sites of TFV drug resistance polymorphisms, using the homologous crystal structure of HIV RTas a model.

The sequence alignment of HBV was extended with HIV RT's p66 domain and then projected onto a high-resolution HIV RT structure (PDB code 3dlk). Sub-domains of the HIV RT are coloured and annotated. Positions associated with resistance are scattered primarily throughout the finger and palm subdomains of the p66 domain (purple space-filled representations, left whole-molecule view,purple stick representation on the zoomed in view on the right). Three aspartate residues, D83, D205 and D206 (indicated by grey space- filled representation) form the catalytic triad of the enzyme and are shown as a point of reference. Of the 37 sites identified as potential TFV RAMs, 24 residues which are visible in the structure are labelled (using HBV numbering). This excludes seven putative HBV mutations at sites which do not have a homologous site in the HIV structure (sites 78, 80, 130, 134, 153, 163 and 256), and six sites which are beyond theend of the sequence of the solved crystal HIV structure (267, 269, 278, 317, 333 and 337). Figure produced using the ICM platform. Notethat in most cases, individual mutations are unlikely to be sufficient to mediate resistance, and a resistant phenotype arises only as a result of combinations of 2 polymorphisms. HBV, hepatitis B virus; HIV, human immunodeficiency virus; RAM, resistance-associated mutation; RT,reverse transcriptase; TFV, tenofovir. common HBV RAMs but does not include positions 78, 177, or 249 which may be pertinent to TFV resistance [39] and 'geno2pheno hbv' only lists one TFV mutation at position 236.

Overlap of TFV RAMs with RAMs to other NA agents RAMs L180M, M204I/V and A181T/V have been associ-ated with resistance to 3TC, telbivudine (LdT) and entecavir(ETV)[3{40-43]. their reported association with TFV resistance is ofconcern in suggesting that prior NA exposure can increase thelikelihood of cross-resistance to TFV. A study of HIV/HBVco-infected individuals demonstrated a decreased likelihood ofHBV DNA suppression with TDF among individuals exposedto prolonged 3TC treatment, possibly due to accumulation of such mutations [44]. A large study in China reported A181 and/or N236 substitutions in 11% of the population [42], which mayunderpin reduced susceptibility to TFV. The structural similari-ties between ADV and TFV, and similar interaction with HBVpolymerase [1,2] explain why the ADV RAMs A181T/V andN236T are also reported to confer resistance to TFV [1,42].

Although TFV has been considered effective in the contextof resistance to other NAs [36], the current evidence suggeststhat there may be common pathways to resistance [37]. Thereis some evidence showing co-location of RAMs conferring resistance to different antiviral agents on the same viral haplotype [38]. These findings suggesting cross-resistance are of concern, especially for settings in which there has been widespread use of NA therapy as a component of ART for HIV [3].

Sites of TFV RAMs in HBV RT

Resistance to TFV can be explained by RAMs both within and outside the active site of the RT enzyme, some of which mayhave similar mechanisms to those described in HIV [10,38]. The mechanism of resistance in most of these polymorphisms remains unknown, but may interfere with drug access to sites of activity through steric hindrance. Mutations within active sitesof the enzyme may be associated with a higher fitness cost tothe virus than mutations at other locations in the RT sequence,as they are more likely to interfere with the RT function. Some polymorphisms listed as RAMs may in fact represent compensatory mutations, which are co-selected in the pres- ence of primary RAMs. For example, substitution at position 269 has been previously described as a compensatory mutation that restores impairments to RT function [39].

Currently, HBV genotyping is not routinely undertaken in clini-cal practice, so it is difficult to amass data for any potential relationship between resistance and viral genotype. However, there are some clues that genotype may be relevant. For example, C256S has been linked to TFV resistance, but S256 is wild typein genotype C (Suppl Table 4, Extended data[5]), suggesting that the genetic barrier to TFV resistance in genotype C mightbe lower than in other genotypes. However, a study of >1000 individuals in China found no differences in drug resistance rates between genotype B vs genotype C infection [42] Theidentification of Y9H as a TFV RAM should be viewed with caution as H9 is frequently the wildtype residue, irrespective of genotype.

Other factors associated with persistent vireamia In addition to RAMs, there are other explanations for incom-plete suppression of HBV viraemia on therapy [22,33], includinga higher baseline HBV DNA level, positive baseline HBeAg status, history of 3TC exposure, a lower nadir CD4+ T cell countin the context of HIV coinfection, and high serum HBV RNA levels[44,40,41]. Given that HBV DNA is inhibited in a dose-dependent manner [2], it is also possible that insufficient drug delivery to the infected hepatocyte could be the cause of persistent viraemia even in the absence of specific RAMs.

Incomplete adherence to therapy can also contribute to virological breakthrough [42]. Two studies included in our review assessed treatment compliance by measuring drug concentra- tion in plasma [9,31]. Assessment of adherence in chronic HBV has been through the use of questionnaires [43], but these are subject to self-reporting bias. Evidence of potential TFV resist- ance may emerge when individuals with HIV/HBV coinfectionare treated with a TFV-containing regimen leading to suppression of HIV but with sustained HBV vireamia [46]

It has been reported to take three years for 90% of HBV infected individuals to reach viraemic suppression on therapy [55], in contrast to HIV, in which 88% of patients suppress the viruswithin the first year of TDF-based treatment [56]. In the studies we have reported in this review, persistent HBV viraemia on therapy could be due to the prolonged timeline for viraemic suppression; however, in most studies there was a reduction in viral load when TDF was initiated, with subsequent virological breakthrough that is more in keeping with the selection of resistance.

Caveats and limitations

There is sparse literature on HBV resistance to TFV, and stud-ies are of varying quality. While there is a high genetic barrier to selection of TFV resistance, it is likely that there is under-reporting of cases of resistance, particularly in low/middle income set- tings in which routine monitoring of HBV viral load on treatment is not undertaken. It can be difficult to infer the impact of common polymorphisms on drug resistance phenotype; forexample, it is plausible that M204I/V may be enriched amongTFV resistant strains simply as a 'footprint' of prior exposureto 3TC.

Most studies to date have used Sanger sequencing, and itis possible that significant minority variants may be under- represented, as suggested by one report in which phenotypic TFV resistance was associated with RAMs in <20% of minorvariants [33[. Low HBV DNA viral loads are a further barrierto sequencing, and bias existing data towards samples from individuals with high viral loads, in which the full spectrum of relevant RAMs may not occur. It is therefore important to invest in deep sequencing platforms that offer the opportunity to explore the full landscape of HBV variants isolated from an infected individual, and to improve sensitivity of sequencing methods including both Sanger and 'next-generation' approaches. Some sequencing methods, such as Oxford Nanopore Technologies, can generate long reads that allow reconstruction of completeviral haplotypes, providing improved certainty about linkages between sites [57]. To be able to undertake an appropriate haplotypes analysis, datasets with robustly phenotyped patients (displaying clinical evidence of drug resistance), together with full length viral sequence data, would be required; such datasets have not been generated to date but are an importantlong-term aim.

We recognise the limitations of drawing direct comparisons between HIV and HBV RT, given the limited (<30%) sequence homology between the two enzymes, and the finding thatonly 2/37 sites associated with TVF resistance in HBV are homologous RAMs in HIV. This highlights a need for futurework to solve the crystal structure of HBV RT.

HBV treatment agents

The full-scale treatment of chronic hepatitis B began in the early 1990s when interferon was first used (5). Then, after the first FDA approval of Lamivudine (LMV) in 1998, nucleo(t)side analogs such as Adefovir (ADV), Entecavir (ETV), Telbivudine (LdT), Clevudine (CLV), and Tenofovir (TDF) came to be used worldwide (Table 1) (6). Despite the various advantages of interferon (IFN-alpha), it is inconvenient to use as an injection and is of limited use for patients with decompensated cirrhosis; thus, orally administered antiviral agents are currently the mainstream treatment for chronic hepatitis B. As tenofovir alafenamide fumarate (TAF) and besifovir dipivoxil maleate (Besifovir, BSV) were approved as a treatment medication for adults with chronic hepatitis B in South Korea in 2017, a total of eight antiviral agents can now be used (Figure. 2) [7-15].al. 2018)

Drug	mechanism	Dose	company	USA status	Development of resistance	Considerations for use	Monitoring during treatment
Epivir (Lamivudine)	Inhibits reverse transcriptase activity of HBV polymerase	Oral, 100 mg/d	GlaxoSmithKline (GSK), UK	Approved 1998	24~30% after 1 year, and 70% after 5 years	-	HBV DNA and serological tests every 3-6 months
Hepsera (Adeforvir dipivoxil)		Oral, 10 mg/d	Gilead Sciences, USA	Approved 2002	20~29% after 5 years	-	
Baraclude (Entecavir)		Oral, 0.5~1.0 mg/d	Bristol-Myers Squibb, USA	Approved 2005	· 1.2% after 5 years in naïve patients · Over 50% in LMV resistance patients	Recommended as first-line therapy	
Tyzeka (Telbivudine)		Oral, 600 mg/d	Novartis, USA	Approved 2006	5% after 1 year, 25% after 2years		
Viread (Tenofovir)		Oral, 300 mg/d	Gilead Sciences, USA	Approved 2008	Low resistance		
Vemlidy (TAF or tenofovir alafenamide)	Prodrug of tenofovir	Oral, 25 mg/d	Gilead Sciences, USA	Approved 2016	No long-term follow-up data available		

Table 1: HBV treatment drugs available for chronic Hepatitis B virus (HBV) infection (Modified from Tang LSY et

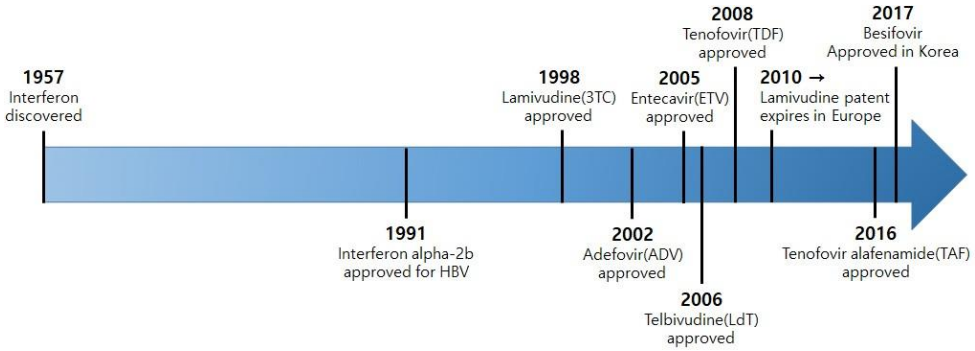


Figure 2: Advances in HBV treatment.

Lamivudine (LMV, 3TC)

LMV was originally developed for AIDS treatment as an inhibitor for HIV reverse transcriptase, and was also approved as a hepatitis B treatment drug, i.e. a 1st-generation nucleotide analogue, by the FDA in 1998 because it effectively inhibits HBV reverse transcriptase (7). LMV is successively phosphorylated to LMV triphosphate by intracellular kinase, and shortly after the diphosphate groups are eliminated, LMV 5'-monophosphate is incorporated into the newly produced viral DNA byHBV polymerase. Because it is a nucleoside analog without a 3'-OH group for chain polymerization, it induces the termination of polymerization synthesis and inhibits viral replication [16]. Although the action mechanism of most antiviral agents is similar to this, drug-resistant mutation within five years is as high as 70%.

Adefovir (ADV)

ADV, an adenosine monophosphate analogue, is phosphorylated by intracellular kinase and activated to ADV diphosphate, whereupon it competitively inhibits the reaction deoxyadenosine triphosphate, a substrate of HBV DNA polymerase. ADV diphosphate is incorporated into newly produced viral DNA, and has an antiviral effect caused by a similar action mechanism to that of LMV, but the recommended dosage is 1/10 (10 mg/day) that of LMV, and its rate of drug-resistant mutation within 5 years has been improved by 20-29% (6). Although ADV had an important role for LMV-resistant patients, it is no longer recommended as a first-line therapy [17-18].

Entecavir (ETV)

ETV, a guanosine nucleoside analogue approved by the US FDA in March 2005, is known to show the more enhanced drug effect (0.5~1 mg/day) compared to other existing drugs, is less likely to cause an adverse reaction, and has only a 1.2% incidence of drug-resistant mutation within five years (8). Because of this excellent resistance rate, ETV is recommended asa first-line therapy for chronic HBV (9).

Telbivudine (LdT)

LdT, a thymidine nucleoside analogue, is an unmodified L-isomer of thymidine, a naturally occurring nucleoside. Thus, thephosphorylation reaction to the active form LdT triphosphate occurs easily. However, LdT has not been used for a first-linetherapy [18,19] because of the higher frequency of resistance in early time during administration similar to LMV [9].

Clevudine (CLV)

CLV approved in 2006 in South Korea, a pyrimidine analogue (30 mg/day), is known to not only inhibit DNA-dependent DNA activity for HBV polymerase but also to demonstrate an antiviral effect by interrupting reverse transcription and priming. CLV is about 10-fold more potent than LMV against HBV[19] in cell culture, and HBV DNA level was reduced by 2.5 to3 $\log_{10}$ copies/mL for 4 weeks trial with 10 to 200 mg/day (10). CLV is being marketed in Korea and Philippines named as Lenovir and Revovir, respectively.

Tenofovir (TDF)

Tenofovir disoproxil fumarate, a prodrug of Tenofovir (TDF), is an oral antiviral agent approved for HIV and HBV treatments(11). TDF is successively phosphorylated by intracellular kinase and activated to tenofovir diphosphate, and then competeswith endogenous nucleotide deoxyadenosine 5'-triphosphate (dATP) for incorporation into the newly replicated HBV DNA by HBV polymerase. Incorporated TDF, instead of endogenous nucleotide, is a nucleotide analogue without a 3'-OH group,which is required for the elongation of DNA base chains, and thus it induces the termination of polymerization synthesis and inhibits viral replication (12) [20,21] This action mechanism is very similar to that of another nucleotide analogue, ADV, but the antiviral efficacy of TDF is much stronger than that of ADV because the latter is used in a limited dosage (only 10 mg) so as to reduce the development of nephrotoxicity, whereas TDF is used in a far higher dose of 300 mg (13). Also, it is assumed that the higher binding affinity of TDF to HBV polymerase, compared to ADV, is related to its strong efficacy (14). Drug-resistant mutation was not reported initially, but mutations have been observed more recently (6, 15).

Besifovir (LB80380)

Besifovir is an acyclic nucleotide phosphonate similar to Adefovir or Tenofovir. It is a nucleotide analogue and a prodrug of guanosine triphosphate nucleotide analogue LB80317. Besifovir is absorbed into the intestine and deacetylated to the intermediate metabolite LB80331 by esterase in the intestine and the liver, and then oxidized to the active metabolite LB80317 by oxidase (aldehyde oxidase or xanthine oxidase). In the liver cells it is phosphorylated to diphosphate and triphosphate forms, after which it competes with dGTP to bind with HBV DNA polymerase, and thus blocks the action of polymerase and inhibits viral proliferation. After the phase 2 clinical trial conducted. It was released on the market in 2017 as a domestic novel drug, witha similar efficacy to that of TDF, but without the latter's adverse side effect of reduced bone density (16) [22].

Tenofovir alafenamide fumarate (TAF, GS7340)

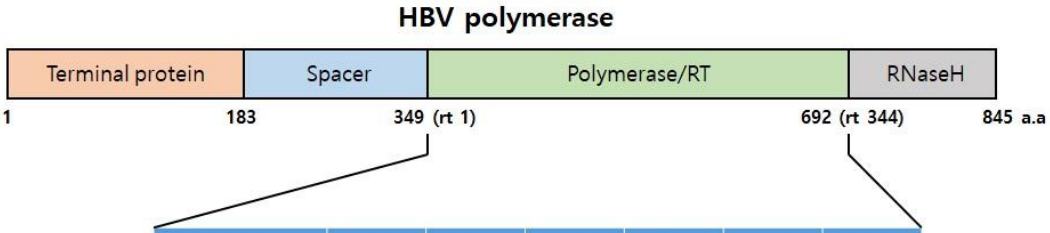
TAF, a prodrug of Tenofovir used in the body and a nucleotide analogue like TDF, is an oral antiviral agent that inhibits reverse transcription from pre-genomic RNA to DNA. Prodrugs are being developed to reduce its adverse effects on the functions of the kidneys by increasing bioactivity and enhancing the antiviral action compared to the strong antiviral agent TDF. The most representative drug is TAF. TAF is converted to tenofovir-alanine conjugate (TAF-Ala) in the body and thenconverted again to TDF and phosphorylated to active metabolite tenofovir diphosphate (TAF-DP) for drug action. Recently, the results of a phase 3 clinical trials for comparison with TDF in HIV patients showed similar antiviral effects, but TAF showed significantly better responses compared with the disadvantages of

TDF, such as increased serum creatine, proteinuria, and reduced bone density. In addition, studies on CMX157, a hexadecyloxypropyl conjugate of TDF, and on AGX-1009 [23] of another structure, are currently in progress.

HBV drug resistance

For the effective treatment of viruses, drugs are administered by monotherapy or combination therapy. Methods with the least incidence of resistant viruses and a quick treatment for HBV have been widely studied and are currently being appliedin clinical situations.

The long-term administration (i.e. more than one year) of a medication for chronic HBV infection [24] leads to the development of drug-resistant mutation viruses in many cases. This is because there is an active site in the center of HBV polymerase where DNA synthesis occurs; if mutations of the amino acids inherent in each drug occur near this site, drugs cannot incorporate into the site due to steric hindrance, whereupon resistance develops. Ultimately, it is most important to select the optimal antiviral agent for treatment by continuously monitoring each drug for the development of a mutation after its administration, because each drug displays different resistance patterns and mutations during long-term administration. The characteristics of resistance for each antiviral agent studied up to the present time are as follows (Fig. 3).



	LMV	ADV	ETV	LDT	CLV	TDF
L80V/I	○			○		
L129M					○	
I169T			○	○		
V173L	○				○	
L180M	○		○	○		○
A181T/V		○				
T184L/G			○	○		
A194T						○
S202G/I			○	○		
M204I/V	○		○	○	○	○
L229V					○	
N236T		○				
M250I/V			○	○		
H337N					○	

Figure 3: HBV resistance to nucleo(t)side analogues.

Lamivudine resistance

The incidence of resistant viruses during LMV treatment has increased by 14~32% each year, and the drug shows high resistance of about 80% after 48 months (17). The primary resistance mutation for LMV is the YMDD[25] motif of the Cdomain of HBV polymerase. It is an rtM204I/V mutation in which YMDD is changed to YIDD or YVDD. In addition, mutations L80V/I, V173L, and L180M have been observed found in the RT areas.

Adefovir resistance

The incidence of ADV resistance is known to be lower than that of LMV. In HBeAg-negative patients, resistance develops inabout 2% of patients after two years of ADV monotherapy, but resistance has been observed in almost 30% of patients after five years of monotherapy. HBV RT domains in rtA181T/V and rtN236T are known ADV resistance mutations (18). In an in vitro drug susceptibility assay, rtN236T mutation did not affect the susceptibility for LMV, LdT, and ETV, but rtA181T mutation reduced the susceptibility for LMV (< 10-fold), ADV (< 2-8 fold), and TDF (< 2-3 fold) [26].

Entecavir resistance

ETV shows the strongest antiviral effect among the drugs currently being used. No resistance was observed initially, and itwas observed in less than 1% of patients during the two-year period (19). Also, it is effective in LMV-resistant rtL180M andrtM204V (20). Until now,

ETV resistant mutations have been found in the B domain (rtI169T, rtL180M, rtT184L), C domain (rtM204I/V, rtS202G/I), and E domain (rtM250I/V). However, Entecavir resistance does not cause cross resistance in ADV or TDF[27].

Telbivudine resistance

The primary resistance mutation of LdT is known to be rtM204I/V, often accompanied by rtL80I/V and rtL180M. The frequency of resistance after two years of therapy is lower than for LMV but higher than for ADV or ETV. In an in-vitro drugsusceptibility experiment, rtM204I, rtL180M/rtM204V, rtI169T/rtM250V, and rtT184G/rtS202I were shown to be mutations for resistance (21). Because LdT is an L-nucleoside analogue, LdT resistance does not cause cross resistance with ADV, TDF or ETV, and thus is used as an alternative treatment for resistance to those drugs (22).

Clevudine resistance

The primary resistance mutation of CLV includes rtM204I and the recently discovered rtL229V as a compensatory mutation for this. Also, the quadruple mutation of rtL129M+rtV173L+rtM204I+rtH337N, which exhibits very strong resistance, to both CLV and LMV (23), has been discovered [28,29].

Tenofovir resistance

TDF is known to be the drug with the least resistance. In TDF treatment of patients with both HBV and HIV infection, thertA194T mutation was found along with the rtL180M + rtM204V mutation, but in an in vitro experiment using a cell line,the rtA194T mutation

showed partial resistance to TDF regardless of the rtL180M+rtM204V mutation (about 5~7 times)(24). A treatment-naïve patient showed the TDF-resistance at rtY9H, rtL91L, rtS106C, rtH126Y, rtD134E, rtQ267L, rtI269L,
rtA317S, rtK333Q and rtN337H, but the clinical significance remains unclear (25).

Besifovir resistance

In a multi-centered phase 2b clinical trial conducted to compare the treatment effect of BSV (90 mg or 150 mg) and ETV

0.5 mg, a viral breakthrough was observed at 16 weeks only in 1 patient with lower drug compliance among the 31patients to whom BSV 90 mg was administered, but no viral mutation related to BSV resistance was observed [26].

Tenofovir alafenamide fumarate resistance

TAF, like TDF or ETV, is classified as a drug with a high genetic barrier, and thus is expected to show an identical genetic barrier as TDF [27, 28]. Additional studies and accumulated clinical data are needed to understand the mutant virus, its appearance, and its influences.

Research background and goals

Nucleo(t)side analogs such as LMV, ADV, ETV, and TDF are currently being used to inhibit HBV replication for the treatment of chronic hepatitis B, but long-term administration of these drugs leads to the development of drug resistance, making a complete cure impossible; thus it is urgent to develop drugs that target various parts of the life cycle of HBV. At the KNIH, studies are under way to investigate the cccDNA regulation mechanism for complete cure of HBV. To identify the potential antiviral effects by discovering the intracellular host factors, candidate antiviral molecules are tested for the inhibition of cccDNA formation.

Research content

Host factors that regulate the MAPK (mitogen-activated protein kinase) signaling system related to HBV replication were discovered and their influences on HBV life cycle were studied. For this, a hepatoma cell line was manufactured in which the discovered host factors can be stably expressed. Then, it was observed that the secretion of HBV antigens (HBeAg, HBsAg), DNA replication, RNA, and protein expression decreased. Also, the inhibition of mRNA and protein expression of the transcription factor HNF4α (hepatocyte nuclear factor 4 alpha) that binds to the HBV enhancer was observed. In conclusion, it was identified that the discovered host factors inhibit the activity of the HBV enhancer and suppress HBV transcription. As such, the chemical compounds related to this have been selected and their HBV inhibition effect is now being analyzed. It is considered that the results of this study will provide the scientific basis for the development of a novel treatment for hepatitis B (drug repositioning) without resistance and which targets the various life cycles of HBV.

Conclusion

There is emerging evidence for polymorphisms that may reduce susceptibility to TVF. However, good correlation between viral sequence and treatment outcomes is currently lacking; further studies are essential to optimise individual treatment and public health approaches. Currently used antiviral drugs for HBV are viral polymerase inhibitors. LMV has mainly been used as an initial treatment drug for chronic hepatitis B, while ADV has been used as an alternative for LMV resistance. As shown in Fig. 3, the long-term use of HBV treatment drugs can induce drug-resistant mutation within the active site of the reverse transcriptasedomain of HBV. Compared to the antiviral agents such as ADV, ETV, and LdT, TDF has been used because these are strong agents with less resistance even during long-term administration, but further studies are necessary whether recently reported mutations are clinically significant. Currently available drugs are unable to achieve the complete eradiation of the cccDNA of chronic hepatitis B, resulting to an inactive carrier state with suppressed viral replication and continuous surveillance for liver cancer. Therefore, it is essential to develop antiviral agents with a totally different action mechanism targeting the HBV life cycle, for example, inhibiting the HBV entry to hepatocytes, the HBx interaction, HBV core assembly, and HBV entry to the hepatocytes (29). Also, new immunomodulatory therapies targeting HBV such as Toll-like receptor agonists have been developing for overcoming the host tolerance.

It is no doubt that nucleo(t)side is the first-line therapy, but occurring the mutations during long-term administration are limitation for complete cure of HBV. Further studies on a combination therapy using a nucleo(t)side analogue and novel targeting antiviral agents to HBV life cycle and immune response should be conducted for cure the HBV without the adverse effects.

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