Hepatitis delta virus: From infection to new therapeutic strategies

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Abstract

The hepatitis delta virus (HDV) is a small RNA virus that encodes a single protein and which requires the hepatitis B virus (HBV)-encoded hepatitis B surface antigen (HBsAg) for its assembly and transmission. HBV/HDV co-infections exist worldwide and show a higher prevalence among selected groups of HBV-infected populations, specifically intravenous drug users, practitioners of high-risk sexual behaviours, and patients with cirrhosis and hepatocellular carcinoma. The chronic form of HDV-related hepatitis is usually severe and rapidly progressive. Patterns of the viral infection itself, including the status of co-infection or super-infection, virus genotypes (both for HBV and HDV), and persistence of the virus' replication, influence the outcome of the accompanying and manifested liver disease. Unfortunately, disease severity is burdened by the lack of an effective cure for either virus type. For decades, the main treatment option has been interferon, administered as mono-therapy or in combination with nucleos(t)ide analogues. While its efficacy has been reported for different doses, durations and courses, only a minority of patients achieve a sustained response, which is the foundation of eventual improvement in related liver fibrosis. The need for an efficient therapeutic alternative remains. Research efforts towards this end have led to new treatment options that target specific steps in the HDV life cycle; the most promising among these are myrcludex B, which inhibits virus entry into hepatocytes, lonafarnib, which inhibits farnesylation of the viral-encoded L-HDAg large hepatitis D antigen, and REP-2139, which interferes with HBsAg release and assembly.

Key Words: Hepatitis delta virus; Hepatitis B virus; Myrcludex; Lonafarnib; REP 2139

Core Tip: The hepatitis delta virus (HDV) is a small defective virus, of interest to scientists for its replicative reliance on and ability to inhibit the common hepatitis B virus (HBV). HDV infection occurs worldwide but shows some geographic variation and its prevalence is generally underestimated. HDV/HBV co-infection causes severe liver disease and the persistence of viral replication is associated with poor prognosis, although interferon is effective in around a quarter of patients. Improved understanding of the HDV life cycle has led to identification of specific antiviral targets and new drugs, including inhibitors of the sodium tauro-cholate cotransporting polypeptide receptor and farnesyltransferase.

INTRODUCTION

The hepatitis delta virus (HDV) is a defective virus that survives in the host by enveloping itself with the hepatitis B surface antigen (HBsAg) provided by the hepatitis B virus (HBV)[1]. This unique virus was discovered in 1977 by Rizzetto *et al* [2] following description of the delta antigen in HBsAg carriers. The subsequent research uncovering HDV modes of replication and its interactions with HBV have provided information with clinical implications[3]. HDV is highly infectious and induces a heterogeneous liver disease that rapidly progresses to cirrhosis; the outcome itself is influenced by several viral and host factors, including virus genotypes and level of viremia (for both HDV and HBV) as well as the presence of any concomitant causes of liver damage[3]. According to recent reports, 10.58% of general HBV-positive individuals have co-infection with HDV, excluding the intravenous drug users (IVDUs) and practitioners of high-risk sexual behaviours (HRSBs)[4].

Collectively, these data highlight the need for HDV screening and control strategies, which must be developed rationally. In the industrialized world, the decline of new infections has regrettably reduced awareness and testing for HDV[1,5]. Moreover, there is a significant lacuna in the literature, with limited data reported from the Americas, Southern Africa and Asia[4], although reliable data from across the globe are required to overcome this pathogenic threat. Once HDV infection is diagnosed, the greatest challenge to clinical care is related to the treatment of associated liver disease. Interferon (IFN) treatment has been the only pharmacological option for decades but its efficacy remains unsatisfactory[6]. The perseverance of HDV researchers is beginning to pay off, however, with enhanced knowledge on the viral life cycle leading to development of drugs targeting specific steps in such, several of which are now under advanced clinical evaluation[6-8].

VIROLOGY

HDV is similar to viroids and virusoids in its structure and replication process but it presents a distinctive identity. Accordingly, HDV was classified as the sole member of the *Deltaviridae* genus[1,9]. The virus contains about 1700 nucleotides and has a circular structure that is formed by base-pairing involving 74% of its genomic RNA. It encodes a single protein - the delta antigen (HDAg) — which combines with the HDV genome to form a ribonucleoprotein complex (referred to as an RNP). The RNP becomes enveloped upon interaction with HBV, which serves as a helper virus, providing the HBsAg component. The latter is necessary for HDV assembly, release and transmission, but it is not required for any of the other steps in replication[3,10]. HDAg is present in two isoforms: small (S-HDAg) and large (L-HDAg), each carrying out a specific role in the HDV life cycle. S-HDAg is composed of 195 amino-acids and promotes RNA replication, while L-HDAg is composed of 214 amino-acids and is essential for the virion packaging. The isoforms differ by 19 amino-acids located in the C-terminal region of L-HDAg exclusively, the presence of which results from an

editing process that is regulated by the adenosine deaminase enzyme ADAR1[11]. HDAg modification occurs at the post-transcriptional level, similar to the well-known processes of phosphorylation, acetylation, methylation and isoprenylation, all of which regulate the hepatitis viral life cycles (Figure 1).

HDV replicates exclusively in the liver, inside hepatocyte nuclei. The virus uses the same entry mechanism as HBV, being enveloped by HBsAg, differentiated in the small (S-HBsAg), large (L-HBsAg) and medium (also known as M-HBsAg) forms. After ligating the hepatocyte's surface heparan sulphate proteoglycans (commonly referred to as HSPGs), the pre-S1 domain of L-HBsAg binds to the receptor for HBV, identified as the sodium tauro-cholate cotransporting polypeptide (NTCP)[12]. The viral RNP then translocates into the nucleus, where its genomic RNA is transcribed first into the complementary antigenomic RNA, then into new genomic RNA and mRNA by the host's RNA polymerase II enzyme. The fact that HDV does not encode its own RNA polymerase enzyme but redirects the host's is a further peculiarity of this hepatitis virus.

Once linear polymers are produced in the HDV-infected hepatocyte, they self-cleave *via* a ribozyme activity of the virus and then circularize. The viral mRNA then migrates from the nucleus to the cytoplasm, for subsequent synthesis of either S-HDAg or L-HDAg. The protein products associate with the viral genomic RNA to form new ribonucleoproteins, each of approximately 20 nm in diameter. This complex relies on HBV surface antigens for complete formation and subsequent secretion[13]. The latter process occurs only after a farnesylation process[14], which makes molecules more lipophilic, whereby L-HDAg interacts with HBsAg at the endoplasmic reticulum.

While at first evaluation this entire process appears rudimentary, HDV has evolved a complex and unusual replicative mechanism, intriguing for researchers but proving a challenge to fully elucidate. It has been proposed, in the field of innovative research, that viruses other than HBV, such as flavivirus and hepacivirus, could package the HDV ribonucleoprotein[15]. This unconventional transmission of HDV certainly requires confirmation in a human system; however, the newest knowledge on its interaction with the NTCP receptor[12] and the farnesylation process of L-HDAg[14] has led to new therapeutic proposals.

EPIDEMIOLOGY

Sero-epidemiological studies performed between the 1980s and 1990s estimated the HDV prevalence among HBsAg-positive patients to be 5%, equating to about 20 million people worldwide[3,16]. Regional prevalence of HDV infection was higher in South Europe, Middle East, East Africa and Asia, with relatively lower rates in Northern Europe, South Africa and North America[17]. During the more recent 2000s and 2010s, the introduction of HBV vaccination, institution of preventive measures and improvements in hygiene habits have led to decreases in HDV prevalence rates in multiple regions. In Taiwan, for example, HDV prevalence among HBsAg-positive subjects declined substantially, from 24% in 1983 to 4% in 1995[18], while in Italy, it declined from 23% in 1987 to 14% in 1992 to 8% in 1997[19]. However, in parallel with the decreased prevalence rates in previously endemic areas, other HDV hot zones emerged, including South Eastern Russia, Northern India, Vietnam and Albania.

Moreover, starting in the early 2000s, immigration activities have prompted a new rise in HDV prevalence in some European States, following a large influx of immigrants from endemic areas, such as Romania, the ex-Soviet Union and North Africa[20]. France also experienced a remarkable increase in cases, reaching a rate of 6.5% in 2010 after remaining stable at around 1% from 1997 to 2005[21]. Similarly, Germany showed an initial decline during the 1990s (from 19% to 7%) but then experienced an upswing trend, with rates fluctuating between 8% and 14%, in the 2000s[22].

Nowadays, HDV infection maintains its worldwide distribution. According to two recent systematic reviews, global prevalence is around 0.16%-0.98% among the general population and 4.5%-14.6% among HBsAg-positive subjects[4,23]. Geographic variations are still present, considering both the overall population and HBV carriers. Among the general population worldwide, Mongolia has reported the highest HDV prevalence[4], with primacy among HBsAg-positive people (prevalence rate of 36.9%). However, prevalence rates greater than 10% are also reported by Moldova and Western and Middle African countries[23]. According to demographical characteristics, men are slightly more affected than women[4] and the elderly are at greater risk
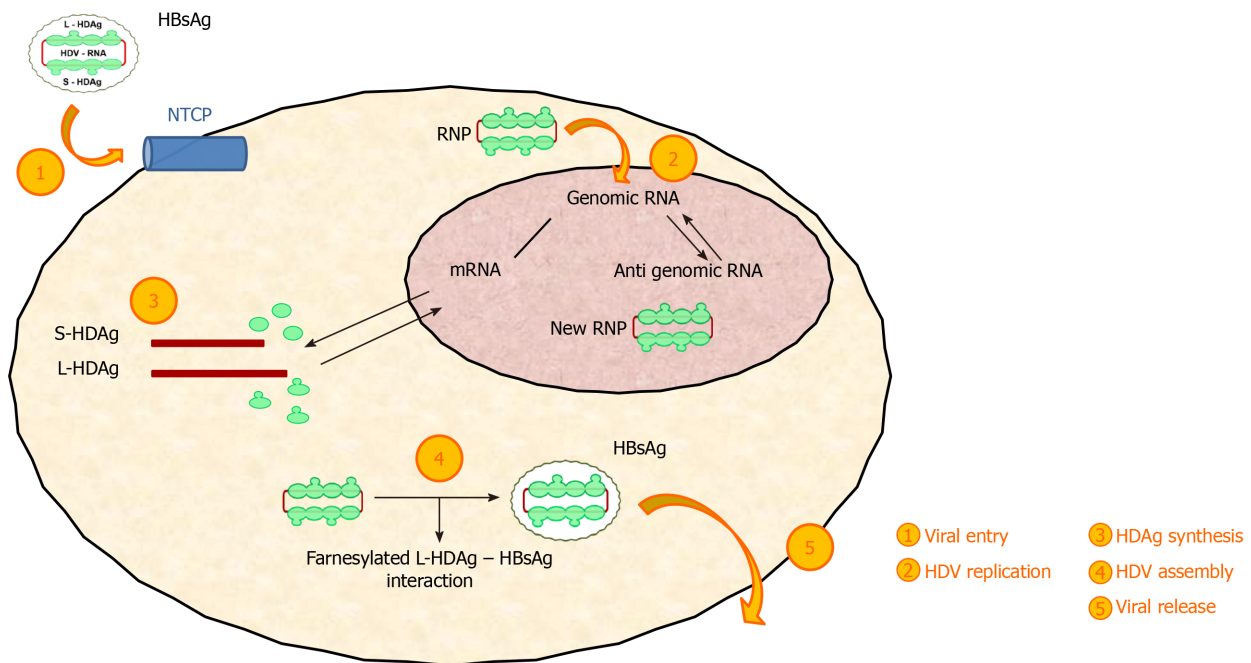
Figure 1 Schematic representation of the hepatitis D virus life cycle. New drugs interfere with the hepatitis B surface antigen at the viral entry or viral assembly level. HBsAg: Hepatitis B surface antigen; HDAg: Hepatitis D antigen; HDV: Hepatitis delta virus; L-HDAg: Large hepatitis D antigen; NTCP: Sodium taurocholate cotransporting polypeptide; RNP: Ribonucleoprotein complex; S-HDAg: Small hepatitis D antigen.

than the young (80% among those ≥ 50-years-old *vs* 3% among those < 30-years-old) [24].

Among the eight recognized HDV genotypes (identified as 1-8), genotype 1 is the most common and it is present worldwide[23,25]. The other seven genotypes are more localized, with genotypes 2 and 4 mainly confined to Asia, genotype 3 to Latin America and genotypes 5-8 to Africa[23].

As exposure to blood of infected subjects is the predominant route of HDV transmission, some groups of high-risk people have been identified. Most HDV-infected patients are IVDUs, who had been infected upon needle-stick injury or use of contaminated syringes[26]. The reported prevalence rates among this subgroup range from 21%[27] to 36%[28], with a pooled odds ratio (OR) of 19.0[23]. Another group of high-risk individuals are practitioners of HRSBs, including commercial sex workers and promiscuous homosexuals[26], among whom the reported prevalence reaches up to 11%[29], with a pooled OR of 18.7[23]. The risk of infection is also higher among the human immunodeficiency virus-positive population (pooled OR: 6.6) and haemodialytic patients (pooled OR: 3.4)[23]. Nowadays, nosocomial infection and transfusion transmission occur less frequently than in the past. However, some cultural practices, such as tattoos and piercing, are quickly becoming an appreciable route of transmission[30].

Taken together, these data show that the global presence of HDV infection has not decreased and it is probably still underestimated.

CLINICAL MANIFESTATIONS AND DIAGNOSIS

Viremia, genotypes and liver disease

The replicative activity of HDV can influence the course of liver disease[31].

A multicentre study found that subjects with persistent HDV replication had worse prognosis in terms of liver failure, need for liver transplantation and/or death than those with undetectable HDV-RNA[32]. On the other hand, the few patients in whom HDV-RNA had become undetectable during their follow-up were determined to be less likely to develop liver cirrhosis than those with persistently positive HDV-RNA levels[32].

To date, little is known about the determinants of spontaneous clearance of HDV-RNA, despite the early clearance of HDV infection being one of the main parameters determining prognosis of the related liver disease[10,33].

Although HBV replication is usually suppressed in the presence of HDV infection, high levels of HBV viremia are associated with more severe liver damage[34]. In addition, HBV genotypes may also play a role in the course of chronic hepatitis D (CHD), which is considered the most severe and rapidly progressive form of chronic viral hepatitis[3].

Besides host-related factors, other viral factors might be involved in clearance of the virus. One such factor is genotypic variability. Among the eight known genotypes, HDV-1 has the highest pathogenic potential[31,33,35], while HDV-2 and HDV-4 are associated with milder forms of the disease[35]. According to a study conducted in Taiwan, patients infected with HDV-2 develop liver failure less frequently than those with genotype 1[34]. HDV-3 has been associated with an increased risk of fulminant hepatitis[3,36], whereas HDV-5 has a better prognosis than HDV-1[33]. The pathogenic properties of HDV 5-8 are not well characterized[7,36]. Among the spectrum of host and viral factors that may underlie the different outcomes of infection with these various genotypes, we can include variability in virus replication and virion assembly efficacy, both of which contribute to the rate of HDV virion secretion, and such host factors as race or presence of single-nucleotide polymorphisms[35].

Clinical manifestation and outcomes of the hepatitis D disease differ according to the HDV acquisition modality, itself depending on HBsAg status of the infected individual. In HBV/HDV co-infections, the presence of both viruses causes wide hepatic necrosis, bringing about severe or occasionally fulminant hepatitis[37]. Acute hepatitis can occur either with a single peak of disease (mono-phasic) or with two distinct peaks (biphasic). In the latter case, the first peak matches to an initial HBV spread, and the second peak to HDV propagation[7,37]. The HBV/HDV co-infection usually leads to a self-limited acute hepatitis; indeed, only 2% of patients with co-infection progress to cirrhosis[3,38-41]. The diagnosis of co-infection is confirmed by the simultaneous presence of serological markers for primary HBV and HDV infections[37]. Generally, the first of these to appear are the host's antibody to hepatitis B core protein (anti-HBc IgM), while that to hepatitis D (anti-HD IgM) appears within 2 wk from clinical onset, usually remaining detectable for up to 5-6 wk afterwards. The anti-HD IgG antibody reaches a detectable level after the IgM antibodies disappear, and may persist for months or even years. A failure in anti-HD IgM clearance predicts the chronicity of hepatitis[26,35,42].

Conversely, in HDV super-infection, the virus infects individuals with chronic HBV infection, and it typically leads to HDV persistence with development of cirrhosis[35]. It may present either as an exacerbation of a known chronic hepatitis B status accompanied by hepatic decompensation, or as a new acute hepatitis status in asymptomatic HBsAg carriers[35]. Super-infection usually leads to a chronic infection (in > 90% of cases), with rapid progression to cirrhosis[23,38-40]. Diagnosis of the super-infection is based upon a positive test for anti-HD IgM and a negative test for anti-HBc IgM in HBsAg carriers[35,37]. The super-infection itself is characterized by early presence of HDV-RNA and HDV antigen, followed by increase in anti-HD IgM that remains persistently detectable, in parallel with development of chronicity. In addition, during the acute phase, there is a suppression of HBV replication[35].

CHD

Among chronic viral hepatitis, CHD is distinguished by its severity and higher risk of evolution. It involves a 3-fold increase in the risk of cirrhosis and a 2-fold increase in the risk of death, as compared to HBV and hepatitis C virus infection alone[3,7,35,39,43]. Half of the patients with CHD have experienced a previous acute hepatitis attack, which often represents the time of super-infection with HDV. Clinically, CHD can range from asymptomatic forms, which are discovered incidentally, to symptomatic ones, manifested as fatigue, malaise, and anorexia[3,35]. Diagnosis is achieved upon detection of high titres of anti-HD IgG and IgM in serum, whereas the HD antigen remains persistently detectable in the liver[35]. Typically, alanine aminotransferase (ALT) levels remain stably elevated or fluctuate, reflecting the destruction of hepatic cells. The lowering of such levels is usually meaningful for tracking disease progression to cirrhosis[35,44].

Cirrhosis

The main complication of chronic hepatitis is the development of cirrhosis. Cirrhosis occurs in 70% of cases within 5 to 10 years after hepatitis development, but in 15% of patients it may occur within 1 year to 2 years[7,37]. While the precise mechanism of this progression remains unknown, it has been proposed that the long form of HDAg may stimulate liver fibrosis through interaction with transforming growth factor-β-induced signal activation[35].

Despite liver biopsy remaining the gold standard procedure for diagnosis and staging of cirrhosis, there are validated (and less invasive) predictive scoring systems applicable to patients with HDV disease. The Delta-4 fibrosis score uses gamma-glutamyl transferase (commonly referred to as GGT), ALT, platelet count, and liver stiffness as parameters[45]. A similar score called the "delta Fibrosis Score" considers GGT along with age, albumin and serum cholinesterase[46]. Abbas *et al*[40] proposed to simply use spleen size and platelet count as the predictive parameters of cirrhosis. Other non-invasive markers of fibrosis that are in use clinically include components of the extracellular matrix, namely procollagen III N-peptide, collagen IV, and hyaluronic acid; however, these factors have lower diagnostic accuracy than in other forms of chronic hepatitis[35].

Once HDV cirrhosis is established, it can remain asymptomatic or induce non-specific symptoms, such as muscle weakness and jaundice. In the advanced stage, it can also evolve complications[37]. In half of the cases, these complications occur within 8 years and include portal hypertension, abdominal ascites, gastrointestinal bleeding and hepatic encephalopathy[31,44]. On average, the annual incidence of liver decompensation in cirrhosis ranges from 2.6% to 3.6%, being more than doubled compared to HDV-negative hepatitis B cases[3,44,47]. Among HDV-positive patients, mortality from cirrhosis and hepatocellular carcinoma (HCC) is higher than that for patients with HBV infection alone[23,44,48].

HCC

HCC is a frequent cause of death from cancer worldwide, and its incidence and mortality rates remain on an upward trajectory. The estimated annual incidence of HCC ranges from 2.6% to 2.8%[26,47]. Chronic viral hepatitis, particularly related to hepatitis B, C and D viruses, is responsible for more than 80% of all cases of HCC[43,49]. While both HBV and hepatitis C virus have already been classified by the World Health Organization as oncogenic viruses, the role of HDV infection in HCC development is still under debate[43,48].

Previous studies have shown that the incidence of HCC was similar among patients with HDV and HBV chronic infections, suggesting that HDV is not a contributory factor, *per se*, to the carcinogenic process. Contrariwise, recent lines of evidence from cohort studies have shown a significantly increased risk of HCC among patients with HDV liver disease, which suggests a greater extent of correlation to HDV than to HBV replication[26,43,48,50,51].

Some studies have estimated that between 16.7% and 20% of cirrhosis or HCC cases among people with hepatitis B worldwide are attributable to HDV infection[23]. Nevertheless, the mechanisms of HCC development in HDV-infected patients remain debated[37]. Bockmann *et al*[48] suggest that the high rate of HCC was caused by the rapid progression to liver cirrhosis and not by viral activity at baseline. In addition, experiments in transgenic mice expressing HDAg proteins seem to rule out a direct carcinogenic role of HDV[3]; however, the L-HDAg may promote oxidative stress through activation of signal transducer and transcription-3 and nuclear factor-kappa B, factors vital to cancer cell communication and carcinogenesis. Moreover, it may also promote cancer cell survival through epigenetic mechanisms, such as DNA methylation of the tumour suppressor gene and acetylation of histone H3 of the clustering promoter[40].

TREATMENT

HDV infection with related liver disease is difficult to treat. Drugs for HBV cure are generally not effective on HDV, and the absence of its own polymerase for the latter complicates attempts to identify suitable therapeutic targets. The ever popular IFN-based therapy was introduced about 35 years ago. Pegylated (Peg)-IFN-α is now preferred over the standard formulation, according to its comparatively improved efficacy and safety. This underlies the biochemical and virological responses that are achieved in about 25% of treated patients[47], with response usually being evaluated clinically at 6 mo post-treatment. Guidelines from the European Association for Study of the Liver recommend Peg-IFN for 48 wk (Table 1) in patients with compensated liver disease and nucleos(t)ide analogues for HBV/HDV patients if HBV replication is above 2000 IU/mL[52]. In the last decade, articles published on the HDV have addressed numerous questions related to the role of combination therapy, to the optimal dose and duration of PEG-IFN administration, and to the real end-points of HDV treatment as well as the clinical benefit.

Table 1 Established and undergoing drugs in the management of hepatitis delta virus chronic hepatitis

Molecule	Drug	Mechanism of action	Drug administration	Duration of therapy	Current therapy/clinical trials	Most frequent side effects
Interferon	Pegylated Interferon-α	Immunomodulatory and antiviral activity	Subcutaneously Weekly	Recommended at least 48 wk	Established Therapy; Long-term follow up, after discontinuation of therapy, available	Flu-like symptoms; Headache; Myalgias; Arthralgias; Anemia; Leukopenia; Thrombocytopenia
Myristoylated Lipopeptide	Myrcludex B; Bulevirtide	Interference with HDV viral entry through NTCP	SubcutaneouslyDaily; ± Peg-IFN; ± Tenofovir	At present maximum 48 wk	Ongoing Phase 3 clinical trial	Thromocytopenia; Neutropenia; Lymphopenia; Eosinophilia; Bile Acids elevation
Farnesyl-transferase Inhibitor	Lonafarnib	Inhibition of HDV viral assembly	Orally; Daily; ± Ritonavir; ± Peg- IFN	At present maximum 48 wk	Ongoing Phase 3 clinical trial	Nausea; Diarrhea; Loss of appetite; Weight loss; Abdominal Bloating Increased ALT levels
Phosphorothioate nucleic acid polymer	Rep 2139	Post-entry inhibition of HBsAg secretion; Possible interference with viral entry	Intravenously; Weekly; ± Peg-IFN	At present maximum 30 wk	Phase 2 clinical trial	Thromocytopenia; Neutropenia; Anaemia; Increased ALT levels

HDV: Hepatitis delta virus; NTCP: Sodium tauro-cholate cotransporting polypeptide; Peg-IFN: Pegylated interferon; ALT: Alanine aminotransferase; HBsAg: Hepatitis B surface antigen.

Strategies to improve the efficacy of Peg-IFN-α

In a large randomized controlled study - the Hep-Net-International Delta Hepatitis Intervention Trial (also referred to as the HIDIT-1) - performed by the German Network, 90 patients were assigned to receive weekly treatment with either 180 μg of Peg-IFN-α plus 10 mg of adefovir (31 patients), 180 μg of Peg-IFN-α plus placebo (29 patients), or 10 mg of adefovir alone (30 patients) for 48 wk[53]. Twenty-four weeks after the treatment initiation, a sustained HDV-RNA clearance was observed in 28% of the patients who had been treated with Peg-IFN-α either as mono-therapy or in combination with adefovir; no therapeutic effect was observed in the group treated with adefovir alone. The HIDIT-2 study[54] embodied a second attempt to clarify if combination therapy was superior to Peg-IFN-α alone in treatment of HDV patients, but this time examining tenofovir use. In that trial[54], 120 patients with HDV-RNA positivity were randomized to receive either Peg-IFN-α plus tenofovir or Peg-IFN-α plus placebo for 96 wk. At the end of therapy, viremia was negative in 48% of patients on combination therapy and in 33% of those on the Peg-IFN-α mono-therapy[54]. At week 24 post-therapy, virological response was observed in less than 30% of patients. The combination therapy with tenofovir did not provide benefits in HDV patients with low HBV-DNA levels, not even when 96 wk of the Peg-IFN-α regimen was compared to 48 wk. However, more side-effects were associated to the prolonged therapy, having consequence for the most advanced form of liver disease.

While other studies have also suggested no clear benefit in extending Peg-IFN-α treatment, positive results were obtained from a retrospective study that included 99 patients, with a median treatment duration of 24 mo and post-treatment follow-up of 55 mo[55]. Altogether, however, the previous studies have recognized the efficacy of IFN in about one-third of treated patients[56]. Yet, the optimal treatment duration remains to be defined and the addition of nucleos(t)ide analogues does not seem to improve the result[57,58].

Predictors of long-term response to IFN

Fifty-six percent of patients showing negativity for HDV-RNA (*i.e.* sustained viral response) in the HIDIT-1 trial experienced a late relapse[59]. These data prompted questions regarding the role of the 6-mo post-therapy negative viremia, considering its potential roles as a surrogate marker of HDV cure and sustained viral response[60]. New suggestions arose from a further sub-analysis of the HIDIT-1 trial to investigate positive and negative predictors of response[60]; in particular, the negative level of HDV-RNA at week 24 of treatment or at the end of treatment was found to identify responders with a prediction value of 71% or 100% respectively. Moreover, HBsAg kinetic parameters during treatment were proposed for monitoring response to

therapy, in association to the HDV-RNA. According to an Italian study, a cut-off of HBsAg < 1000 at month 6 of Peg-IFN therapy is able to discriminate responders and partial responders from non-responders[61]. Furthermore, the association of HBsAg reduction (0.105 Log) and HDV-RNA decrease (1.610 Log) from baseline to month 6 was found to be predictive of HBsAg clearance.

Clinical impact of IFN-based therapy

An important end-point of HDV therapy is HDV-RNA clearance, as determined by sensitive assays[62]. Unfortunately, the presence of minimal residual virus that falls below the threshold of detection of an assay could explain reactivation in the presence of HBsAg. The ideal goal of therapy in HBV/HDV co-infection is the clearance of HBsAg; although, this is rarely achieved[63]. In any case, IFN treatment was reported effective in altering the disease progression-associated events[64]. The loss of HDV-RNA during follow-up was a more frequent occurrence among the IFN-α treated patients and it was linked to long-term survival without complications[55,65]. Based on this observation, a durable undetectability of HDV viremia was proposed as a surrogate and more realistic end-point[64].

New therapeutic targets in HDV

Some new promising drugs have advanced in phase II clinical trials. Specifically, these represent inhibitors of the NTCP receptor[66] and farnesyltransferase[67] and the group of nucleic acid polymers[68] (Table 1).

Myrcludex B: Myrcludex B (MyrB, subsequently named bulevirtide) is a myristoylated synthetic peptide, containing 47 amino acids of the preS1 domain of the HBV L-surface protein. It is able to bind to the NTCP, which has been identified as a receptor for HBV/HDV entry (Figure 1)[12]. The concentration of this drug required to inhibit the virus is 100 times lower than that required for bile salt transport inhibition[69].

After a preliminary study[70], Bogomolov *et al*[71] assessed the efficacy and safety of MyrB in a total of 24 patients. The study design assigned 7 patients to receive the 48-wk Peg-IFN-α treatment, 7 patients to receive 2 mg MyrB administered by subcutaneous injection for 24 wk followed by Peg-IFN for 48 wk, and 7 patients to receive MyrB and Peg-IFN for 24 wk plus an additional 24 wk of Peg-IFN. Notably, the HDV-RNA levels decreased by at least one log in the MyrB cohort at week 24 and ALT normalized in 6 patients. While the decline of viremia was observed in all three treatment groups, HBsAg level showed no change in any.

A subsequent phase II trial enrolled 120 patients with CHD, and randomized them into four treatment groups. Three groups were treated with three different doses of MyrB (2 mg, 5 mg and 10 mg) in combination with tenofovir (245 mg/d), while one group was administered tenofovir alone[72]. At the end of treatment, the best result (a -2.7 Log decline of HDV-RNA) was found in patients who received the higher dose of MyrB plus tenofovir. However, for all groups, about two-thirds of the patients who were responders experienced relapse during the follow-up.

A new phase II study was designed to evaluate the efficacy of 2 mg and 5 mg MyrB combination therapy with Peg-IFN-α, and compared to Peg-IFN-α and MyrB monotherapies[73]. The MyrB and Peg-IFN combination yielded high rates of off-treatment HDV-RNA suppression and reduction in HBsAg. Recently, MyrB was authorized as an orphan medicine by the European Medicine Agency, making it available for usage in patients with HDV compensated liver disease and active viral replication.

Lonafarnib: Lonafarnib (LNF) is a farnesyl-transferase inhibitor, originally developed in cancer treatment. In the HDV setting, the drug inhibits the farnesylation of L-HDAg, which is essential for interaction with HBsAg in the assembly of new viral particles. In a first, short-term controlled study, patients received oral LNF at 100 mg or 200 mg twice daily. While the drug was effective in reducing virus levels, it was mostly associated with gastro-intestinal adverse events and weight loss[74].

Four different subsequent trials were designed and identified as 'LOWR-HDV'. In studies 1 and 2, the LNF was administered at different doses, either alone or in combination with ritonavir or Peg-IFN. Overall, it induced a significant viral decline [75], with a mean log reduction of -5.57 Log^{10} U/mL achieved in the triple combination group[76]. In study 3, patients on nucleos(t)ide analogues were treated with LNF at 50 mg/d, 75 mg/d, 100 mg/d plus ritonavir for 12 wk or 24 wk[77]. The regimen of LNF 50 mg plus ritonavir once daily was superior, when compared to the higher doses of the drug. Finally, the regimen of dose escalation was tested in study 4, the LOWR-HDV-4[78].

Altogether, the findings from these different LOWR-HDV studies supported the antiviral effect of LNF. Moreover, its combination with ritonavir allowed for the use of lower doses and produced more manageable side effects. At present, a phase 3 clinical study (D-LIVR) is ongoing. This is a randomized placebo-controlled trial comparing 50 mg LNF plus ritonavir twice per day with or without Peg-IFN-α in patients maintained on nucleos(t)ide analogues.

Nucleic acid polymers: Nucleic acid polymers are phosphorothioate oligonucleotides with antimicrobial activity, some of which have shown more specific HBV inhibiting properties (*e.g.*, REP 2139). The antiviral effect seems to be related to various mechanisms, including the inhibition of HBsAg release and assembly and the interaction with both S-HDAg and L-HDAg[79,80]. REP 2139 was tested in 12 patients with CHD at a dose of 500 mg administered intravenously once a week for 15 wk, followed by a 250 mg dose combined with subcutaneous injection of 180 μg Peg-IFN for 15 wk and finally Peg-IFN mono-therapy given for 33 wk[81]. Nine patients, who became HDV-RNA-negative during treatment, remained non-viremic at the end of treatment and showed a mean HDV-RNA decline of 5.34 Log^{10} IU/L. REP 2139 induced a remarkable HBsAg decline (3.5 Log^{10} IU/mL) compared to baseline. The most common side effects experienced by patients during treatment were thrombocytopenia, neutropenia and increased ALT levels. Eleven participants were followed for 3.5 years in the REP 301-LTF study[82] and they showed a long-term safety profile as well as persistent virological control and functional cure.

CONCLUSION

HDV remains an important health problem worldwide. Although the infection map is subject to change due to diffusion of the HBV vaccine, some geographic areas are currently a virus reservoir. Moreover, the real burden of infection appears to be underestimated, both in the general and at-risk populations. It is known that HDV-related liver disease is influenced by replicative activity of the virus, which is crucial in inducing cirrhosis and promoting hepatic decompensation. For this reason, the research for new therapeutic strategies has focused on molecules interfering with the replication cycle of HDV.

These novel drugs have demonstrated antiviral efficacy and tolerability, but there remain open questions to be answered and clarified in long-term treatment and follow-up. MyrB is administered *via* subcutaneous injection. Although it induced a decrease in viral loads, it did not reduce the HBsAg level. On the other hand, MyrB showed a good safety profile, even in some patients with compensated cirrhosis. LNF, on the other hand, has the advantage of oral intake. Moreover, when administered in combination with ritonavir, it was the determinant for reduction of gastrointestinal side-effects. For both drugs, viral reactivation and ALT flares were reported at the end of treatment. REP 2139 requires an intravenous administration. It produced a rapid reduction in HBsAg levels, and not only in HDV viremia, in a lasting way; however, this effect needs to be tested on larger numbers of patients.

In conclusion, MyrB, LNF and REP 2139 represent expected and promising therapeutic options for HDV infection. Further studies are needed to define the utility of combination therapy with IFN.

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