A review on hepatitis D: from virology to new therapies

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Abstract
Hepatitis delta virus (HDV) is a defective virus that requires the hepatitis B virus (HBV) to complete its life cycle in human hepatocytes. HDV virions contain an envelope incorporating HBV surface antigen protein and a ribonucleoprotein containing the viral circular single-stranded RNA genome associated with both forms of hepatitis delta antigen, the only viral encoded protein. Replication is mediated by the host cell DNA-dependent RNA polymerases. HDV infects up to 72 million people worldwide and is associated with an increased risk of severe and rapidly progressive liver disease. Pegylated interferon-alpha is still the only available treatment for chronic hepatitis D, with poor tolerance and dismal success rate. Although the development of antivirals inhibiting the viral replication is challenging, as HDV does not possess its own polymerase, several antiviral molecules targeting other steps of the viral life cycle are currently under clinical development: Myrcludex B, which blocks HDV entry into hepatocytes, lonafarnib, a prenylation inhibitor that prevents virion assembly, and finally REP 2139, which is thought to [...]
A review on hepatitis D: From virology to new therapies

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Hepatitis D virus is a defective virus, dependent on hepatitis B virus for its assembly. Hepatitis D virus infection affects 62–72 million people worldwide. Chronic hepatitis D is the most severe chronic viral hepatitis. Current interferon-based antiviral treatments have dismal efficiency and are poorly tolerated. Host-targeting molecules inhibiting the viral life cycle are currently in clinical development.

Graphical Abstract

Hepatitis Delta Virus

Impact
62-72 million people chronically infected worldwide

Risk of fibrosis, cirrhosis, and hepatocellular carcinoma

Under development: Myrcludex B Lonafarnib REP 2139

Currently available: Interferon-alpha

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Abstract

Hepatitis delta virus (HDV) is a defective virus that requires the hepatitis B virus (HBV) to complete its life cycle in human hepatocytes. HDV virions contain an envelope incorporating HBV surface antigen protein and a ribonucleoprotein containing the viral circular single-stranded RNA genome associated with both forms of hepatitis delta antigen, the only viral encoded protein. Replication is mediated by the host cell DNA-dependent RNA polymerases. HDV infects up to 72 million people worldwide and is associated with an increased risk of severe and rapidly progressive liver disease. Pegylated interferon-alpha is still the only available treatment for chronic hepatitis D, with poor tolerance and dismal success rate. Although the development of antivirals inhibiting the viral replication is challenging, as HDV does not possess its own polymerase, several antiviral molecules targeting other steps of the viral life cycle are currently under clinical development: Myrcludex B, which blocks HDV entry into hepatocytes, lonafarnib, a prenylation inhibitor that prevents virion assembly, and finally REP 2139, which is thought to inhibit HBsAg release from hepatocytes and interact with hepatitis delta antigen. This review updates the epidemiology, virology and management of HDV infection.

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Introduction

Hepatitis D or delta is caused by the hepatitis delta virus (HDV), a human pathogen first identified in 1977 [1]. HDV is a defective RNA virus that does not encode its own envelope proteins and depends on the expression of the hepatitis B virus (HBV) surface antigen (HBsAg) in the same cell to complete its life cycle. HDV can enter hepatocytes not expressing HBsAg and efficiently replicate its genome and express the hepatitis delta antigen (HDAg); however, no secretion of infectious particles occurs. Hepatitis D is hence the result of either an acute coinfection by HBV and HDV or a HDV superinfection of patients chronically infected with HBV.
Chronic hepatitis D (CHD) is arguably the most aggressive type of viral hepatitis and is associated with an increased risk of cirrhosis, liver decompensation and hepatocellular carcinoma (HCC) [2], but the management of HDV has evolved little during the past years. The main treatment remains pegylated interferon-alpha (IFN-alpha), with unsatisfactory results. Nucleos(t)ide analogues specific for HBV have no effect on HDV replication. However, several host-targeting molecules with a specific impact on HDV life cycle are currently under development.

**Epidemiology**

Worldwide, ~248 to 292 million people are chronically infected with HBV [3,4], Based on these estimations, ~15 to 20 million of these patients were initially thought to be also affected by HDV [5]. These figures were challenged by a recent meta-analysis, proposing that a staggering 62–72 million people may live with HDV worldwide [6], a prevalence almost two-times greater than that of human immunodeficiency virus (HIV) infection (estimated to infect 36.9 million persons in 2017, according to the World Health Organization). These estimates imply a disease burden much higher than previously considered and one that is still debated [7]. Indeed, the exact global prevalence of HDV infection remains unknown because of heterogeneous and non-standardised screening practices and the inaccessibility to testing in many endemic areas.

In Mongolia, HDV infects ~60% of the HBsAg-positive individuals, corresponding to the highest reported prevalence worldwide [8]. Other highly affected areas include the Amazon basin [9], West Africa [10,11], the Mediterranean basin [12] and Eastern Europe [13].

In Western Europe, although high prevalence rates were described in Italy early after HDV identification, a subsequent decrease was documented as consequence of improved socioeconomic conditions and mass vaccination campaigns against HBV [14,15]. HDV prevalence now seems to be very low in some European countries, and in close association with intravenous drug use (IVDU) [16]. However, no decrease has been observed in other areas, likely because of migration from endemic regions [17,18].

In the United States, HDV infection has for long been considered rare and screening recommendations are limited to high-risk populations [19]. Unfortunately, several recent studies highlight the presence of suboptimal testing rates and suggest that the prevalence may be much higher that previously considered [20–22].

As HBV, HDV can be transmitted by blood and blood-derived products and sexual contact. Vertical transmission is however rare. In highly endemic populations, transmission occurs mainly through intrafamilial and iatrogenic spread [23] in association with poor hygiene conditions [24]. In low endemicity regions in the northern hemisphere, iatrogenic and intrafamal transmission, while accounting for infections occurred in the past, are no longer common and IVDU is now the main transmission route [6]. Sexual transmission, although less frequent than for HBV or HIV, seems to be important in regions where HBV infection is endemic, such as Taiwan [25,26].

**Virology**

**Classification**

HDV is the smallest known virus infecting mammals, for which humans are the only natural reservoir. Other susceptible mammalian hosts have been identified as well as used for research purposes; these include chimpanzees, tree shrews (both with HBV as a helper virus) and woodchucks (in the presence of the woodchuck hepatitis virus, WHV). Although HBV orthologues have been found in a variety of non-human mammals and have been shown to have potential cross-species infectivity [27], no HDV orthologue had been described until the very recent identification of HDV-like agents in birds and snakes [28,29].

Due to its distinct characteristics, HDV has been postulated to originate from plant viroids or cellular circular RNAs and is currently the sole member of the Deltavirus genus [30–32].

There are eight HDV genotypes, highly heterogeneous, with up to ~40% of sequence divergence [33]. Genotype 1 is present worldwide and is the predominant virus in Europe and North America. Genotype 3 is identified in South America, while genotypes 2 and 4 are common in East Asia and genotypes 5–8 are mainly found in Africa (refer to [34] for a review of genotype distribution). Although good quality studies are limited, different genotypes seem to be associated with distinct liver disease severity. In comparison with genotype 1, genotypes 2 and 4 seem to cause milder liver disease. Genotype 2 in particular, has been associated to a lower incidence of cirrhosis, HCC and decreased mortality than genotype 1 in a prospective study conducted in Asia [35]. Genotype 3, on the other hand, is associated with a more severe course of acute infections and a higher risk of acute liver failure [36].

A co-evolution of HBV and HDV genotypes can be suggested by the frequency of specific genotype pairs, the most commonly reported being the combination of HDV genotype 3 with HBV genotype F [37]. However, these associations have been argued to merely result from geographic distribution, given these are not strict combinations [36] and HDV virion assembly has been shown to be possible with several HBV genotypes [38].

**Viral structure**

HDV circulating virions were firstly characterised in chimpanzees infected with serum from an Italian chronic carrier [39]. As represented in Fig. 1. These 35–37 nm particles are composed of an envelope and a ribonucleoprotein (RNP).

Since HDV is a defective virus and does not code for its own surface proteins, it uses the three forms of HBV surface proteins (small or S-HBsAg, medium or M-HBsAg and large or L-HBsAg) on which it depends to form its own envelope and egress and re-entry into hepatocytes. These proteins share a common C-terminus (S domain, the only constituent of S-HBsAg). M-HBsAg contains an N-terminal hydrophilic domain named PreS2 and, relative to M-HBsAg, L-HBsAg N-terminus consists of an additional domain named PreS1 [40].

The RNP, present both in viral particles and infected cells, contains the viral genome associated with both isoforms of the hepatitis delta antigen (HDag) — small, S-HDag, and large, L-HDag — forming a structure that is essential for the nuclear trafficking of HDV RNA and for viral assembly [41]. Although debate surrounds its complete characterisation, its assembly depends on the oligomerisation of HDAG molecules and the secondary structure of the HDV genome [42–45].

The HDV genome, whose complete structure was first reported in 1986, is a circular, covalently closed, single-stranded RNA of ~1680 nucleotides with 74% internal base pairing, allowing the folding into a partially double-stranded rod-like structure [46].

During HDV replication in the infected cells, two other main forms of viral RNAs can be found: the antigenome, which is a replication intermediate and the exact complement of the genome sequence [47,48], and the HDV mRNA coding for the two isoforms of the HDag [49]. Ribozymes (small, self-cleaving RNA sequences) have been described in both the HDV genome and antigenome and are responsible for the cleavage of the multimeric linear RNA molecules that arise during replication [50].
Viral life cycle

The following paragraphs describe the main steps of the HDV life cycle and Fig. 2 depicts a simplified, schematic version.

Viral entry

HDV is considered to target primarily hepatocytes. Although the possibility of extra-hepatic replication in a natural infection has been hypothesised for HBV, namely in lymphocytes [51,52], no such evidence exists for HDV [53]. However, HDV replication can take place in a wide range of mammalian cells, if the genome is experimentally delivered, suggesting that its hepatotropism depends exclusively on the presence of the receptor [54].

HDV is considered to enter hepatocytes through the same mechanisms as HBV, given that both viruses share a similar envelope [2]. Infectivity of both viruses depends on the presence of L-HBsAg, in particular on the 75 amino acids located at its...
N-terminal (in the PreS1 domain), where an essential myristoylation site is located [40], as well as specific amino acid residues of S-HBsAg. However, M-HBsAg does not seem necessary [55,56].

A first, non-specific step consists in the viral attachment to the heparin sulfate proteoglycans (HSPGs) exposed on the outer face of the host cell membrane [57–59]. One particular HSPG, glypicanc-5, has been identified as an entry factor that only partially justifies HDV and HBV dependence on HSPGs, as its abrogation was not sufficient to completely prevent infection [60].

Attachment, although necessary, is not sufficient to allow viral entry and further interaction of the virus with its specific receptor is needed. For the first three decades following the identification of both HBV and HDV, this receptor was unknown. In 2012, the human sodium taurocholate cotransporting peptide (hNTCP, encoded by SLC10A1) was convincingly shown to be a functional receptor to both HBV and HDV in hepatocytes [61]. This molecule is located at the basolateral membrane of hepatocytes and is involved in the uptake of bile acids. The interaction of the bile acid binding domain of NTCP with the myristoylated N-terminal sequence of the PreS1 region of L-HBsAg was shown to be both necessary and sufficient for HBV and HDV infection [61,62]. The post-entry steps involved in the release of the HDV RNP in the cytoplasm and its transport into the nucleus, where transcription and replication subsequently occur, are not fully characterised.

Replication
HDV replication occurs in the nucleus and is completely independent of HBV. Since HDV does not possess its own RNA-dependent RNA polymerase or use the polymerase of its helper virus, the host cell DNA-dependent RNA polymerases likely mediate its replication. Several lines of evidence support the involvement of RNA polymerase II, but a debate is still ongoing concerning the role of the other cell RNA polymerases in HDV replication [54,63]. Both RNA polymerases I and III have been shown to bind HDV RNA. While RNA polymerase I seems implicated in the antigenomic transcription, no precise function has been reported so far for RNA polymerase III [64].

The mechanisms through which the virus is able to hijack the cell DNA-dependent RNA polymerase(s) for its RNA replication are still largely unknown and, although similar mechanisms have been shown to play a role in plant viroid replication [65], HDV constitutes a unique case in human virology. The secondary structure of HDV RNA might play a role in the capacity of the host RNA polymerase II to use it as template, as the enzyme recognises sites located at the two poles of the rod-like structure of the genome [66]. Arguably, S-HDAg would also play an important role in this process [67]. Indeed, the regulation of viral replication involves both forms of HDAg: while L-HDAg, which is essential for HBV, its release from hepatocytes depends on the presence of HDV infection in the same cell. HDV assembly depends on the specific interaction between the farnesylated N-terminus of L-HDAg and the S region of HBsAg [82], and it has been shown that, unlike HBV, HDV RNP can efficiently be assembled with the small form of HBsAg (S-HBsAg) [83]. However, the large form of HBsAg (L-HBsAg) is needed to form infectious virions (as mentioned earlier, it mediates the interaction between the virus and its hepatocyte receptor, NTCP). The relevance of the non-infectious, S-HBsAg enveloped, HDV particles in a natural infection is still to be demonstrated.

Assembly and release
As mentioned previously, although HDV can replicate and synthesise new RNP independently of HBV, its release from hepatocytes requires the presence of HBsAg in the same cell. HDV assembly depends on the specific interaction between the farnesylated N-terminus of L-HDAg and the S region of HBsAg [82], and it has been shown that, unlike HBV, HDV RNP can efficiently be assembled with the small form of HBsAg (S-HBsAg) [83]. However, the large form of HBsAg (L-HBsAg) is needed to form infectious virions (as mentioned earlier, it mediates the interaction between the virus and its hepatocyte receptor, NTCP). The relevance of the non-infectious, S-HBsAg enveloped, HDV particles in a natural infection is still to be demonstrated.

In the context of a HBV infection, the three forms of HBsAg, which are produced in much higher amounts than required for HBV virion production, can self-assemble and be secreted as “empty” subviral particles (SVPs). These non-infectious SVPs (constituted of an envelope devoid of HBV capsid or nucleic acids) are secreted in large excess relative to the infectious virions and are

RNAs editing and L-HDAg synthesis
A single open reading frame in the HDV antigenome directs the synthesis of both isoforms of HDAg (S-HDAg and L-HDAg). These two isoforms differ by an additional C-terminal stretch of 19 amino acids in L-HDAg. As described before, the replication of HDV RNA requires S-HDAg, whereas L-HDAg is essential for virion assembly. The C-terminal domain of L-HDAg enables the interaction with HBsAg [74]. The relative ratio between these two HDAgs isoforms regulates the equilibrium between replication and virion assembly. The editing of the antigenic RNA by adenosine deaminase acting on RNA 1 (ADAR1) drives the transition from S-HDAg mRNA to L-HDAg mRNA transcription [75]. This cellular enzyme has two isoforms: the small, which is expressed constitutively and the large, whose expression is stimulated by type I IFN. Contradictory results still exist regarding the role of each ADAR-1 isoform in the HDV life cycle [76,77]. ADAR1 acts on a particular site of the HDV antigenome, called the amber/W site. This site is a UAG amber stop codon, leading to translation termination and consequent S-HDAg production. ADAR1 deaminates an adenosine (UAG → UIG) and the resulting inosine is recognised as a guanosine in the next replication cycle, leading to an ACC triplet on the genome (instead of the original AUC). Transcription of this triplet generates a tryptophan-encoding UGG codon in the mRNA that no longer works as a stop codon. Consequently, translation proceeds for an additional 19 amino acids, resulting in L-HDAg synthesis [75], as depicted in Fig. 3.

Post-translational modifications of HDAg proteins
Both HDAg proteins undergo post-translational modifications critical for their respective functions. Phosphorylation of two serine residues of S-HDAg allows interactions with the cellular RNA polymerase II, enabling the replication of HDV RNA [78,79]. A farnesyl signal (C211XXQ box) in the additional 19 amino acids of L-HDAg enables a farnesyl lipid group to be added covalently to the cysteine at position 211 by a cellular farnesyltransferase. This farnesylated form of L-HDAg inhibits the replication of HDV RNA and is essential for virion assembly [74]. Indeed, agents that inhibit the addition of the farnesyl lipid group to the C-terminus of L-HDAg prevent its interaction with HBsAg, consequently inhibiting HDV virion secretion both in vitro and in vivo [80,81] and constitute a novel therapeutic approach for HDV infection (see Drugs in Clinical Development below).
thought to play a role in HBV escape to the immune response. HBV SVPs are secreted through the Golgi, while infectious virions follow the multivesicular body pathway [84]. Given that the composition of the HDV envelope is close to that of SVPs and that titers of circulating HDV virions are higher than those of HBV virions, approaching those of HBV SVPs [2,85], it is likely that HDV uses the SVP secretion pathway for its assembly and release.

Molecular interactions with HBV

Studies conducted in experimental models have shown a decrease in HBV replication in the context of HDV infection, with minimal impact on the expression of HBsAg, as demonstrated by an increased HBsAg/HBV DNA ratio in the HBV-HDV co-infected cells, in comparison with HBV monoinfected cells [86–88]. This observation has been confirmed in patients, although the viral dominance patterns seem to fluctuate over time [89,90].

Several mechanisms may be used by HDV to inhibit its helper virus replication, while ensuring a constant pool of HBsAg for its own assembly. Firstly, the possibility of an epigenetic regulation of cccDNA transcriptional activity by HDAg has been suggested from both in vitro results and patients samples [91], raising the possibility of a differential transcription of PreS/S mRNA vs preregemonic mRNA. Secondly, both isoforms of HDAg have been shown to interact with and strongly repress both HBV enhancer sequences, with a direct impact on HBV replication [92]. Thirdly, HDAg, being an RNA-binding protein that has recently been shown to interact with specific cellular RNAs [93–95], may bind to HBV mRNAs and selectively affect their stability. Finally, accumulating evidence suggests that, in HBV-infected patients, integrated HBV DNA is an abundant source of HBsAg, even in the absence of HBV replication [96–98]. Furthermore, HBsAg derived from integrated HBV DNA has been shown to support assembly and release of infectious HDV particles [99]. While the impact of this mechanism in vivo is still to be demonstrated, it is tempting to hypothesise that HDV can complete its cycle using HBsAg produced from integrated HBV DNA, devoid of HBV replication in the same hepatocyte.

Indirect mechanisms of interference through a deregulation of the hepatocyte innate immune response are also possible. HBV has classically been considered not to be recognised by the innate immune system [100]. While this notion has been challenged by evidence suggesting that the virus may in fact actively counteract the interferon response [101,102], two recent studies support the concept of HBV as a “stealth virus” [103,104]. HDV, on the other hand, has been shown to induce a strong type I IFN response [86,105] as a result of the recognition of viral RNAs by melanoma differentiation antigen 5 (MDA5) [106]. The consequent increased expression of antiviral IFN-stimulated genes (ISGs), such as MxA, may contribute to the inhibition of HBV replication [92].

The interplay between HDV and the host cell IFN response is however far from being clarified. HDV replication is itself inhibited by the administration of exogenous IFN-alpha [86,107]. The mechanisms could involve, among others, the increased synthesis of L-HDAg as a consequence of the stimulation of ADAR1 expression [108]. Furthermore, IFN-alpha has been shown to inhibit HDV propagation during cell division, suggesting yet another antiviral mechanism [109] [Zeng Z et al, 2018 International HBV Meeting]. It is tempting to hypothesise that the virus may have developed mechanisms to resist the strong IFN response induced by its own replication, and HDV has indeed been shown to interfere with the JAK/STAT signalling pathway, a mechanism that might play a role in viral persistence [110].

Clinical presentation and natural history of the disease

Two modalities of HDV infection exist: simultaneous co-infection with HBV and HDV superinfection of a person chronically carrying HBV. Coinfection translates into acute hepatitis, during which aminotransferase levels can follow a typical biphasic course, corresponding to an initial HBV spread followed by HDV propagation. As for HBV monoinfection, in most immunocompetent adult patients (90–95%), it progresses to resolution of both HBV and HDV infections. The risk of acute liver failure is however much higher than that during acute HBV monoinfection [111,112]. Acute
HDV superinfection of a patient chronically infected with HBV is associated with an episode of acute hepatitis that can be mistaken for a HBV flare. In this setting, the risk of acute liver failure is particularly high [111]. More than 90% of HBV carriers superinfected with HDV progress to chronic dual infection [111,112].

CHD is considered the most severe form of chronic viral hepatitis, with a faster progression towards cirrhosis and a higher risk of decompensation and mortality [113]. Indeed, 10–15% of chronically infected patients might develop cirrhosis within 5 years from infection and up to 80% after 30 years [114].

The association between HDV and HCC is still debated. On the one hand, decompensation of chronic liver disease, and not HCC, has been shown to be the most common complication of CHD [115,116]. On the other hand, despite the long-standing belief that HDV-infected patients do not present an increased risk of HCC, several cohort studies have recently found that this risk may indeed be as much as nine times higher than in HBV monoinfected patients [20,113,117,118]. Furthermore, persisting HDV replication was shown to be a risk factor for liver disease progression and HCC [119]. Other factors of disease progression are male sex, cirrhosis at presentation and lack of antiviral therapy [116].

Diagnosis of HDV infection

Recommendations of the major societies currently differ in the screening strategy for HDV diagnosis. European guidelines advise for HDV screening of all HBV-infected patients [120]. In the United States, and despite increasing evidence pointing to a suboptimal diagnosis of HDV infection [20,21], screening is only advised in patients with specific risk factors (including migrants from regions with high HDV endemicity, a history of IDU or high-risk sexual behaviour, individuals infected with HCV or HIV and patients with elevated aminotransferases with low or undetectable HBV DNA) [19]. Given the recent evidence suggesting that the global burden of disease may be higher than previously estimated, screening of all HBsAg-positive patients may be considered. Such a strategy would not only allow a more accurate determination of the prevalence of HDV infection, but it would also lead to wider and earlier therapeutic interventions, decreasing the burden of disease complications. Furthermore, access to care would be significantly strengthened by the implementation of point of care diagnosis.

Several markers can be used for the diagnosis of HDV infection. Anti-HDV antibodies can be detected by enzyme-linked immunosorbent assay (ELISA) or radioimmunoassay (RIA). Anti-HDV total antibody is currently used as a first screening approach for the detection of HDV infection. However, two main limitations should be borne in mind, justifying the need for complementary approaches for diagnosis confirmation. Firstly, total anti-HDV antibody can be undetectable in the early weeks of acute infection. Secondly, anti-HDV IgG may persist after HDV infection, not allowing the distinction between active and resolved infection. A quantitative microarray antibody capture (QMAC) assay for the quantification of anti-HDV IgG has been recently validated for HDV diagnosis in Mongolia and the United States and was shown to correlate with detection of HDV RNA [8,121]. Anti-HDV IgM appears earlier during the acute infection and has been shown to correlate with disease activity during chronic infection [122]; however, it is frequently undetectable in this setting and hence does not allow diagnosis confirmation nor distinction between acute and chronic infection. Serum HDAg is only transiently detected in the acute phase of HDV infection and its measurement is of limited utility.

Infecction confirmation relies on the detection of HDV RNA by quantitative RT-PCR, which, together with a positive anti-HDV antibody, allows to distinguish between chronic and past infections and to follow response to treatment [123]. However, unlike for other viruses, lack of standardisation is still problematic for HDV and PCR results are often not comparable between laboratories [124]. Recent steps have been taken towards standardisation, with the availability of a World Health Organization standard, allowing result reporting in international units (IU) and new pangenotypic commercial assays [125]. In cases where a liver biopsy sample is obtained, intrahepatic HDAg may be detected by immunohistochemistry and HDV RNA by in situ hybridisation. Although determination of HDV genotype is possible by PCR, its use is limited to research settings.

Management of HDV-infected patients

The management of acute hepatitis D relies on general support measures or referral for liver transplantation if acute liver failure develops. No antiviral treatment has proven useful [126]. There are currently no specific direct-acting antiviral treatments for HDV and, although several host-targeting molecules are under development, current recommendations for CHD treatment are limited to a prolonged course of pegylated IFN-alpha.

Patient follow-up and treatment outcomes

The ideal endpoint for CHD treatment would be the clearance of both HBV and HDV infections from the liver, translating into anti-HBs seroconversion, to prevent liver disease progression. Although on-treatment and post-treatment kinetics of serum HDV RNA have been used to monitor treatment response, cumulative evidence exists that, unlike HCV infection, they fail to predict long-term virological outcome. Indeed, although undetectable HDV viremia is expected to be a marker of on-treatment response, a classical definition of sustained virological response (persistently undetectable viral RNA for 24 weeks after treatment) should be used with caution in CHD, as later relapses have been shown to occur in more than 50% of the patients treated with pegylated IFN-alpha [127].

Currently available antiviral treatments

Pegylated IFN-alpha

Although, as described above, its precise mechanism of action still needs to be clarified, IFN-alpha remains the only recommended treatment for CHD [19,120]. Pegylated IFN-alpha, having a prolonged plasma half-life, allows a once-a-week administration, with better efficiency and compliance than standard IFN-alpha [123,128]. Indeed, in a meta-analysis performed in 2011, standard IFN-alpha treatment was associated with a 17% sustained suppression of HDV RNA at six months follow-up (compared to 25% in pegylated IFN-alpha) and with more frequent and severe adverse events (e.g. anorexia, nausea, weight loss, alopecia, leukopenia and thrombocytopenia) [129]. Results are comparable for pegylated IFN-alpha 2a and 2b [130].

Data from clinical trials do not allow an accurate prediction of response and no robust stopping rules exist. However, HDV RNA negativity at 24 weeks of treatment has been identified as a predictor of sustained HDV RNA suppression during follow-up [131]. Nevertheless, optimal treatment duration has not been established. In most studies, pegylated IFN-alpha was used for 48 weeks and this is now the recommended treatment duration. As shown in Table 1, results diverge among studies and considering the variability of PCR performances recently demonstrated [124,132], the reported sustained responses at 24 weeks of post-treatment follow-up may have been overestimated in earlier studies. In one large randomised clinical trial, a 48-week course of pegylated IFN-alpha led to a persistently undetectable HDV RNA 24 weeks after treatment in 25–30% of patients [133]. Shorter treatment
Severity was observed in 60% of patients who achieved undetectable HDV RNA in 53% of them. Furthermore, an improvement in liver fibrosis was demonstrated with pegylated IFN-alpha monotherapy or 24 weeks of Myrcludex B as monotherapy or in combination with pegylated IFN-alpha. A summary of the main studies evaluating the efficiency of IFN-based regimens in HDV infection is provided in Table 1.

A clinical trial tested adefovir as monotherapy or in combination with pegylated IFN-alpha, but none of these treatments showed a better efficacy than pegylated IFN-alpha alone, and adefovir treatment alone had no effect on HDV viremia. The same result was later observed with tenofovir. However, a prospective South American study reported encouraging results with the combination of entecavir and pegylated IFN-alpha for 48 weeks in patients infected with HDV genotype 3, as 21 of the 22 patients included had an undetectable HDV RNA level at the end of treatment and at the six months follow-up, and 20 of 22 had undetectable level of HBV DNA at the six months follow-up, suggesting that genotype 3 might react differently to these molecules, potentially being easier to treat. Finally, a study conducted with HIV co-infected patients treated with tenofovir for 58 weeks showed a good response with no detectable levels of HBV DNA in all patients and with no detectable levels of HDV RNA in 53% of them. Furthermore, an improvement in liver fibrosis severity was observed in 60% of patients who achieved undetectable HDV RNA levels. Although this improvement may be a mere consequence of the immune reconstitution resulting from antiretroviral treatment, a benefit of prolonged therapy with NUCs in CHD cannot be excluded.

Drug resistance to NUCs is emerging, as HDV replication is dependent on the host cell RNA polymerases for its replication and may be more difficult to treat as HDV has no helper virus. The alternative strategies currently being developed are based either on the indirect stimulation of the innate immune system (as is the case of IFN-lambda) or of cell targets involved in other steps of the viral life cycle as entry (Myrcludex B) and viral assembly and release (lonafarnib and REP 2139). A summary of the most relevant results obtained in recent clinical trial results is presented in Table 2.

In summary, NUCs act on the HBV reverse transcriptase, and efficiently inhibit HBV replication, with little effect on HBsAg expression. Although in theory inhibiting the helper virus is expected to affect HDV life cycle, in reality, NUCs are not effective against HDV. Molecules tested in HDV infection include famciclovir, ribavirin (in combination with pegylated IFN-alpha), lamivudine, and entecavir, but none demonstrated effectiveness.

As HDV depends on the host cell RNA polymerases for its replication, and even though alternative viral targets as the ribozyme could eventually be inhibited, the development of antiviral molecules that directly and specifically target this step has not been successful. The alternative strategies currently being developed are based either on the indirect stimulation of the innate immune system (as is the case of IFN-lambda) or of cell targets involved in other steps of the viral life cycle as entry (Myrcludex B) and viral assembly and release (lonafarnib and REP 2139). A summary of the most relevant results obtained in recent clinical trial results is presented in Table 2.

**Table 1**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Treatment modalities</th>
<th>Number of patients</th>
<th>Sustained suppression of HDV RNA at 24-weeks of follow-up</th>
<th>References</th>
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<tr>
<td>IFN-alpha: 3–18 Mio units 3x/week</td>
<td>3–12 months</td>
<td>201</td>
<td>17%</td>
<td>[129]</td>
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<td>Pegylated IFN-alpha 2b: 1.5 μg/kg qw</td>
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<td>16</td>
<td>25%</td>
<td>[137]</td>
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<td></td>
<td>18 months + Ribavirin (1–1.2 g qd for 12 months)</td>
<td>22</td>
<td>18%</td>
<td>[137]</td>
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<tr>
<td>Pegylated IFN-alpha 2a: 180 μg/kg qw</td>
<td>12 months</td>
<td>14</td>
<td>43%</td>
<td>[123]</td>
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<tr>
<td>Pegylated IFN-alpha 2a: 180 μg/kg qw or Pegylated IFN-alpha 2a: 180 μg/kg qw</td>
<td>12 months</td>
<td>12</td>
<td>17%</td>
<td>[138]</td>
</tr>
<tr>
<td>Pegylated IFN-alpha 2a: 180 μg/kg qw or Pegylated IFN-alpha 2a: 180 μg/kg qw</td>
<td>12 months + adefovir (10 mg qd for 12 months)</td>
<td>29</td>
<td>26%</td>
<td>[133]</td>
</tr>
<tr>
<td>Pegylated IFN-alpha 2a: 180 μg/kg qw or Pegylated IFN-alpha 2a: 180 μg/kg qw</td>
<td>12 months + adefovir (10 mg qd for 12 months)</td>
<td>31</td>
<td>31%</td>
<td>[133]</td>
</tr>
<tr>
<td>Pegylated IFN-alpha 2a: 180 μg/kg qw or Pegylated IFN-alpha 2a: 180 μg/kg qw</td>
<td>12 months + adefovir (10 mg qd for 12 months)</td>
<td>104</td>
<td>23%</td>
<td>[140]</td>
</tr>
</tbody>
</table>

**Abbreviations:** qw, weekly; qd, daily.

**Nucleoside/nucleotide analogues**

Nucleoside/nucleotide analogues (NUCs) act on the HBV reverse transcriptase, and efficiently inhibit HBV replication, with little effect on HBsAg expression. Although in theory inhibiting the helper virus is expected to affect HDV life cycle, in reality, NUCs are not effective against HDV. Molecules tested in HDV infection include famciclovir, ribavirin (in combination with pegylated IFN-alpha), lamivudine, and entecavir, but none demonstrated effectiveness.

A clinical trial tested adefovir as monotherapy or in combination with pegylated IFN-alpha, but none of these treatments showed a better efficacy than pegylated IFN-alpha alone, and adefovir treatment alone had no effect on HDV viremia. The same result was later observed with tenofovir. However, a prospective South American study reported encouraging results with the combination of entecavir and pegylated IFN-alpha for 48 weeks in patients infected with HDV genotype 3, as 21 of the 22 patients included had an undetectable HDV RNA level at the end of treatment and at the six months follow-up, and 20 of 22 had undetectable level of HBV DNA at the six months follow-up, suggesting that genotype 3 might react differently to these molecules, potentially being easier to treat. Finally, a study conducted with HIV co-infected patients treated with tenofovir for 58 weeks showed a good response with no detectable levels of HBV DNA in all patients and with no detectable levels of HDV RNA in 53% of them. Furthermore, an improvement in liver fibrosis severity was observed in 60% of patients who achieved undetectable HDV RNA levels. Although this improvement may be a mere consequence of the immune reconstitution resulting from antiretroviral treatment, a benefit of prolonged therapy with NUCs in CHD cannot be excluded.

**Pegylated IFN-lambda**

IFN-lambda is a type III IFN with structural features, receptor characteristics and biological activities that are distinct from IFN-alpha, while sharing common ISC induction pathways associated with its antiviral activity. It has been shown to have an antiviral effect against HDV comparable with IFN-alpha in humanised mice. In patients with chronic hepatitis B, the administration of IFN-lambda in a pegylated formulation led to virological outcomes equivalent to those of pegylated IFN-alpha, but with a better tolerability, which makes it a potentially attractive option for the treatment of CHD. It is currently being evaluated in phase II clinical trials both in monotherapy (NCT02765802) and in combination with lonafarnib and ritonavir (NCT03600714).

**Myrcludex B**

Myrcludex B, a myristoylated lipopeptide, inhibits the entry of HBV and HDV in hepatocytes. Its sequence corresponds to the N-terminal amino acids (2–48) of L-HBsAg and inhibits viral entry by binding to its natural receptor, NTCP, at the basolateral membrane of hepatocytes. Data from preclinical studies indicate that the antiviral effect can occur without interference with the bile acid transport function of NTCP. Indeed, while bile acid transport can be affected by high doses of Myrcludex B (IC50 47 nmol/L), effective viral entry inhibition can be achieved at much lower doses (IC50 80 nmol/L).

In a phase Ib/IIa trial, 24 patients with CHD received standard pegylated IFN-alpha monotherapy or 24 weeks of Myrcludex B as monotherapy or in combination with pegylated IFN-alpha. Although no changes in HBsAg levels (the primary endpoint) were observed, the serum HDV RNA levels were significantly reduced (1.67 log10 in the Myrcludex B group, 2.6 log10 in the Myrcludex B plus pegylated IFN-alpha and 2.2 log10 in the pegylated IFN-alpha monotherapy arm). While some patients achieved undetectable HDV RNA levels at the end of treatment, viral rebound was universal after treatment cessation.
Table 2
Summary of the studies evaluating molecules in clinical development.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Treatment duration</th>
<th>Number of patients</th>
<th>Virological outcome Development stage and References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pegylated IFN-lambda</td>
<td>120 or 180 μg qw sc</td>
<td>48 weeks 33</td>
<td>At week 24 of treatment: 4/10 patients are HDV PCR-negative Phase 2 [148]</td>
</tr>
<tr>
<td>Myrcludex B 2 mg/kg qd sc, 24 weeks followed by pegylated IFN-alpha monotherapy, 48 weeks</td>
<td>72 weeks 24</td>
<td>Decline in HDV RNA at week 24 of treatment: 1.67 log10 decrease in HDV RNA Phase 2 [149]</td>
<td></td>
</tr>
<tr>
<td>Myrcludex B 2 mg/kg qd sc + pegylated IFN 24 weeks followed by Pegylated IFN-alpha monotherapy, 24 weeks Pegylated IFN-alpha monotherapy</td>
<td>48 weeks</td>
<td>Decline in HDV RNA at week 24 of treatment: 2.17 log10</td>
<td></td>
</tr>
<tr>
<td>Myrcludex B 2, 5 or 10 mg qd sc</td>
<td>24 weeks 120</td>
<td>Decline in HDV RNA at week 24 of treatment: 2 mg: 1.75 log10 5 mg: 1.6 log10 10 mg: 2.7 log10 Phase 2b [150]</td>
<td></td>
</tr>
<tr>
<td>Tenofovir 245 mg qd po</td>
<td>24 weeks</td>
<td>Decline in HDV RNA at week 24 of treatment: 0.18 log10</td>
<td></td>
</tr>
<tr>
<td>Myrcludex B 2 or 5 mg qd sc + pegylated IFN-alpha sc</td>
<td>48 weeks 30</td>
<td>Decline in HDV RNA at week 48 of treatment: 2 mg: 3.62 log10 5 mg: 4.48 log10 Phase 2 [151]</td>
<td></td>
</tr>
<tr>
<td>Myrcludex B 2 mg qd sc</td>
<td>48 weeks 15</td>
<td>Decline in HDV RNA at week 48 of treatment: 2.84 log10</td>
<td></td>
</tr>
<tr>
<td>Pegylated IFN-alpha sc</td>
<td>48 weeks 15</td>
<td>Decline in HDV RNA at week 48 of treatment: 1.14 log10</td>
<td></td>
</tr>
<tr>
<td>Lonafarnib 100 or 200 mg bid iv</td>
<td>4 weeks 14</td>
<td>Decline in HDV RNA at day 28 of treatment: 100 mg: 0.73 log10 200 mg: 1.54 log10 Phase 2A [152]</td>
<td></td>
</tr>
<tr>
<td>Lonafarnib 200 mg bid po</td>
<td>12 weeks 3</td>
<td>Variation in HDV RNA at week 12 of treatment: 0.03 log10 Phase 2 [153]</td>
<td></td>
</tr>
<tr>
<td>Lonafarnib 300 mg bid po</td>
<td>12 weeks 3</td>
<td>Decrease in HDV RNA at week 12 of treatment: 1.78 log10</td>
<td></td>
</tr>
<tr>
<td>Lonafarnib 100 mg tid po</td>
<td>5 weeks 3</td>
<td>Decrease in HDV RNA at week 4 of treatment: 1.31 log10</td>
<td></td>
</tr>
<tr>
<td>Lonafarnib 100 mg bid po + pegylated IFN-alpha qw sc</td>
<td>8 weeks 3</td>
<td>Decrease in HDV RNA at week 8 of treatment: 2.19 log10 Decrease in HDV RNA up to 3.7 log10 at week 24 of treatment</td>
<td></td>
</tr>
<tr>
<td>LNF 100 mg po bid + ritonavir 100 mg qd po</td>
<td>8 weeks 3</td>
<td>Decrease in HDV RNA at week 8 of treatment: 2.97 log10</td>
<td></td>
</tr>
<tr>
<td>Lonafarnib 50 mg bid po (increased at 4 week intervals to 75 mg and then 100 mg) + ritonavir 100 mg bid po</td>
<td>24 weeks 15</td>
<td>Dose escalation possible in 10 patients At the end of treatment, mean HDV RNA decline was 1.38 ± 1.38 log10 IU/mL. Phase 2 [154]</td>
<td></td>
</tr>
<tr>
<td>Lonafarnib 50, 75 or 100 mg qd + ritonavir 100 mg qd po</td>
<td>12 or 24 weeks 21</td>
<td>Decrease in HDV RNA at week 12 of treatment: 50 mg: 1.6 log10 75 mg: 1.3 log10 100 mg: 0.83 log10</td>
<td></td>
</tr>
<tr>
<td>Lonafarnib 50 mg bid po + ritonavir 100 mg bid po</td>
<td>24 weeks 33</td>
<td>Decrease in HDV RNA at week 24 of treatment: 21 of 33 patients had a &gt; 2 log10 decrease in HDV RNA Phase 2 [156]</td>
<td></td>
</tr>
<tr>
<td>REP 2139-Ca 500 mg qw iv 15 weeks followed by REP 2139-Ca qw + pegylated IFN-alpha 15 weeks followed by pegylated IFN-alpha 33 weeks</td>
<td>63 weeks 12</td>
<td>- At week 30 of treatment: &gt;5 log decline in HDV RNA in 11 patients Undetectable HDV RNA in 10 patients - At the end of treatment: HBs seroconversion in 5 patients; Undetectable HDV RNA in 9 patients; – 18 months after treatment: 4 patients HBsAg negative 7 patients maintain undetectable HDV RNA Phase 2 [157,158]</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: bid, twice a day; iv, intravenous; po, per os; qw, weekly; qd, daily; sc, subcutaneous, tid, three times per day.
A recent uncontrolled trial included 12 patients that were treated with REP 2139-Ca in combination with pegylated IFN-alpha (patients received REP 2139-Ca only for 15 weeks, followed by a combination of REP 2139-Ca and pegylated IFN-alpha for 15 weeks and finally pegylated IFN-alpha only for 33 weeks) [157]. At the end of combination therapy (week 30), 10/12 patients had undetectable HDV RNA and 9/12 patients had HBsAg declines > 2log10 from baseline, 6 of whom had HBsAg seroconversion. At the end of treatment 9 patients remained HDV RNA negative with 6 still having HBsAg seroconversion. Eighteen months after removal of treatment, HDV RNA was still negative in 7 patients and HBs seroconversion was still present in 4 patients [158].

Elevations of aminotransferases were documented in nearly 50% of patients [157]. However, aminotransferases normalized in these patients during follow-up and no other alterations in liver function were documented (with the exception of one patient with bilirubin elevation). Although the results are overall promising, larger phase III trials are required before establishing the efficacy and safety of this treatment.

A long-term follow-up study in CHB is currently under way (with encouraging results presented in 2018 [158]) and a clinical trial is planned to evaluate REP 2139-Mg (once-a-week subcutaneous administration) in combination with tenofovir and pegylated IFN-alpha.

**Vaccination/ prevention**

HBV vaccination protects effectively against both HBV and HDV infection. Vaccination campaigns have indeed reduced the reservoir of HBV patients that can be potentially infected by HDV. A study published in 2007 [163] showed a clear correlation between the introduction of vaccination for HBV and the decrease in HDV incidence particularly among those 15–24 years old, probably also because of reduced iatrogenic transmission. Countries with high HDV endemicity, such as Brazil and Mongolia, have adopted universal HBV vaccination programmes, with an expected impact on the absolute number of new infections. No perspectives for a vaccination strategy to prevent HDV infection in HBV-infected patients currently exist, as results in animal models have been discouraging [164].

**Conclusions and future perspectives**

Hepatitis D is considered the most severe form of chronic viral hepatitis. It currently has no satisfactory treatment and a better understanding of its pathogenesis is warranted. HDV infection is highly endemic in resource-limited countries, where clinical trials are difficult to conduct and, while it is considered infrequent in developed countries, its real prevalence may be underestimated. Thanks to significant advances in the characterisation of the viral life cycle, several host-targeting molecules are currently in clinical evaluation with promising results.
Conflict of interest

The authors have declared no conflict of interest.

Compliance with Ethics Requirements

This article does not contain any studies with human or animal subjects.

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