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Abstract

Background: Therapy of chronic hepatitis D (CHD) is still based on interferon (IFN) alfa, introduced in clinical practice 30 years ago: results are modest and better therapies are an urgent medical need.

Objectives: This article provides a critical overview of the new therapies under investigation for CHD.

Sources: Review of the recently published medical literature.

Content: New therapeutic efforts aim to deprive the Hepatitis D Virus (HDV) of functions provided to its life cycle by the Hepatitis B Virus (HBV) or by the host. Three therapeutic strategies are in evaluation: 1) Myrcludex B, a myristolated lipopeptide of the pre-S1 domain of the HBsAg that blocks the entry of the HDV into hepatocyes and controls infection by preventing the spreading of the virus to liver cells not infected by the HBV; 2) Lonafarnib, an inhibitor of a host farnesyl-transferase that hinders morphogenesis of the HDV by preventing the farnesylation of the large HD-antigen, necessary for virion assembly; 3) REP 2139, a nucleic acid polymer that prevents export of the mature HDV by the presumed inhibition of the synthesis of subviral HBsAg particles with which the virion is coated. Myrcludex B and Lonafarnib increase therapeutic efficacy in combination with Peg-IFN alfa. In a pilot study, REP 2139 in combination with Peg-IFN alfa induced the clearance of serum HDV RNA and of the HBsAg in about half of 12 treated patients.

Implications: Long-term therapies with either Myrcludex B or Lonafarnib in combination with Peg-IFN alfa are required to achieve clinical control of CHD. However, with prolonged therapies tolerance becomes a problem; studies are on the way to determine whether Peg-IFN lambda may be better tolerated that Peg-IFN alfa. The promising preliminary data of REP 2139 in combination with Peg-IFN alfa await confirmation of the original pilot study.

Keywords: Hepatitis D, Hepatitis D Virus, HBsAg infection, viral liver disease, antiviral therapy.

Introduction

Chronic Hepatitis D (CHD) is the most severe form of viral hepatitides, rapidly leading to to cirrhosis, liver dysfunction and hepatocellular carcinoma [1].

Infection with Hepatitis D Virus (HDV) was estimated to occur worldwide in 15 to 20 million out of the 300 million chronic carriers of the Hepatitis B Virus (HBV), but its medical burden may be significantly higher; a recent metanalysis has calculated that the global prevalence of HDV among HBsAg carriers is as high as 13.02%, corresponding to 48-60 million infections [2].

There is yet no efficient treatment for HDV infections. Therapy still relies on interferon alfa (IFNα) which was empirically introduced in clinical practice more than 30 years; the efficacy is poor and the addition of antivirals against the partner HBV, like Adefovir (ADV), Entecavir (ETV) and Tenofovir (TDF), is of no avail [3]. In the largest trial of CHD, the Hep-Net International Delta Hepatitis Intervention Trial (HIDIT-1) the cumulative rate of sustained viral response (clearance of serum HDV mantained six months after stopping treatment) was 28% using Pegylated (Peg)-IFN either in monotherapy or in combination with ADV [4]; however, relapses were frequent post-therapy [5].

New therapies against hepatitis D are an urgent need but the challenge is daunting. With a RNA genome of only about 1700 nucleotides, the HDV does not code for proteins like the viral polymerases and proteases of the HBV and Hepatitis C Virus; it depends for dissemination and replication on the helper HBV and the host replicative machinery [6], and cannot therefore be targeted by conventional antivirals, like those currently used to control the HBV or cure Hepatitis C.

Targeting host factors critical to viruses replication is an emerging approach in the treatment of infectious diseases and current efforts against Hepatitis D are addressed at disrupting the viral life-cycle by depriving the HDV of critical biological functions provided to it by the liver cell.

New therapeutic agents

Three novel therapeutic strategies have been devised (Fig. 1).

1) The entry into hepatocytes of HBV/HDV entails the binding of the HBsAg to the Sodium Taurocholate Co-transporting Polypeptide (NTCP) located on the cell membrane [7].

Drugs interfering with the ingress of the HBsAg may provide a therapeutic option. Of the agents that inhibits the cellular binding of HBsAg in vitro [8], the first that entered clinical evaluation is Myrcludex B (Myr) [9], a small peptide that blocks the engagement of the HBsAg preS1 with the NTCP. The rationale is that the blocking of the HBsAg should prevent the de-novo infection of yet uninfected liver cells, leading over time to the elimination of all the HDV-infected hepatocytes and to the recolonization of the liver with regenerating infection-free cells.

2) The assembly of HDV virion requires the farnesylation by the host of the large HD antigen of the virus [10]. Interference with this process by inhibitors of cellular farnesylation leads to the disruption of viral assembly; the farnesylation inhibitor Lonafarnib (LNF) provides the first drug with a human pedigree to disrupt this process [11].

3) The HDV needs to encapsidate in the HBsAg coat for export to blood. Nucleic Acid Polymers (NAP) appear to prevent the synthesis of subviral HBsAg particles [12] and may provide a therapeutic option; REP 2139 is the first NAP used to hinder the release of HDV virions from cells.

Initial studies

Three initial studies have provided proof to the concept of targeting extrinsic processes in the life cycle of the HDV.

In a first Myr study [13], 24 patients received the drug daily by subcutaneous injections for 24 weeks, alone or in combination with Peg-IFNα. The mean HDV RNA decline from baseline was $-1.67 \log_{10}$ IU/mL, $-2.6 \log_{10}$ IU/mL and $-2.2 \log_{10}$ IU/mL in patients given, repsectively, Myr alone, Myr plus Peg-IFNα, and Peg-IFNα alone therapy. In two patients given Myr alone and in 5 given Myr with Peg-IFNα, serum HDV RNA diminished below the lower limit of quantification.

In a first NAP study [14], 500 mg intravenous REP 2139 was given once weekly for 15 weeks to 12 patients in Moldova followed by 250 mg intravenous REP 2139 in combination with Peg-IFNα once weekly for 15 weeks, followed by Peg-IFNα monotherapy once weekly for 33 weeks. After 1 year of follow-up, therapy resulted in the clearance from blood of both HBsAg and HDV RNA and in the raising of high titers of anti-HBs, in nearly 50% of patients.

In a first LNF study [15], 14 patients were randomized into two groups given oral LNF for 28 days. Six of a group of eight patients received LNF 200 mg daily and two received placebo. Four of a group of six patients received LNF 400 mg daily and two received placebo. After completion of therapy, the two patients who received placebo were moved to group 2 and received LNF 400 mg in open-label treatment. By the end of therapy, HDV RNA had declined by a mean of -0.73 $\log_{10}$ IU/ml with the lower dose and by -1.54 $\log_{10}$ IU/ml with the higher dose of LNF. There were important gastrointestinal side effects during therapy; with the 400 mg dosage, 50% of patients had intermittent vomiting and all lost weight.

Follow up studies

While the three initial reports were published in extenso in the international medical press, further therapeutic developments for each drug have been reported mostly in abstract form; complete details are not yet available.

In the first publication, the combination of REP 2139 with Peg-IFN had radical efficacy in 50% of the patients, clearing both the HDV RNA and the HBsAg. However, these striking results were not followed yet by other CHD series; further information on the therapeutic potential of this treatment is eagerly awaited.

In follow-up to the initial LNF report, several studies under the acronym LOWR HDV (LOnafarnib With Ritonavir for HDV) were addressed to determine the optimal protocol for LNF therapy. Since LNF is metabolized by the Cytochrome P450-3A4, the CPY3A4 inhibitor Ritonavir is now added in order to diminish adverse effects while maintaining drug exposure [16].

The LOWR HDV studies have shown that LNF/Ritonavir performs best with the combination of Peg-IFN; the latest protocols make use of Peg-IFN lambda (λ) instead than Peg-IFNα, based on data suggesting a better tolerability [17] and different kinetics [18]. Preliminary information is available from an ongoing study [19] of 26 patients treated with oral LNF 50 mg and Ritonavir 100 mg twice daily together with Peg-IFNλ 180 µg weekly for 24 weeks. TDF or ETV was started prior to therapy. In 19 patients evaluated at the end of therapy, the median HDV RNA decline was 3.4 $\log_{10}$ IU/mL, with 7 patients (37%) achieving undetectable HDV RNA.

More extensive data were reported in follow-up to the first Myr study. In the MYR202 trial [20], 120 patients pretreated with TDF were randomized to TDF alone or TDF with 2 mg, 5 mg, or 10 mg Myr daily for 24 weeks. At end of treatment, serum HDV RNA levels were significantly reduced in the groups receiving Myr but not in the TDF alone group; almost half of the Myr patients normalized ALT versus less than 7% of those given TDF alone.

In the 203 trial [21], 4 groups of 15 patients each received for 48 weeks Peg-IFNα alone or a combination of 2 or 5 mg Myr daily with Peg-IFNα, or 2 mg Myr daily alone; they were followed up off treatment for other 24 weeks. The primary endpoint was undetectable serum HDV RNA at week 72. The HDV RNA was undetectable at end of therapy in 19 of the 60 treated patients but relapsed during the follow up in 7. At week 72 the mean HDV RNA $\log_{10}$ change was higher with combination treatments (- 4.04 and -1.48 logs for combination with 2 and 5 mg Myr) than the monotherapies with Peg-IFNα and 2 mg Myr (-0.26 and -1.08 logs, respectively). ALT remained normal in 1, 7, 5 and 3 of the 15 patients treated, respectively, with Peg-IFNα monotherapy, 2 mg Myr + Peg-IFNα, 5 mg Myr + Peg-IFNα and 2 mg Myr monotherapy. HBsAg became undetectable in 4 of the 15 patients given 2 mg Myr + Peg-IFNα.

In an extension of the 203 Myr study [22], 2 groups of 15 patients each were treated for 48 weeks; one received 10 mg Myr once daily in combination with Peg-IFNα, the other received 5 mg Myr twice daily in combination with TDF. Also in the study, serum HDV RNA levels diminished consistently in both arms given Myr; at week 24, the viral marker had declined by a median -4.84

log$_{10}$ IU/ml from baseline in the Myr/Peg-IFNα group and by -2.80 log$_{10}$ IU/mL in the Myr/TDF group; it became undetectable in 60% of the former and in 21.4% of the latter. Alanine amino-transferase (ALT) normalized in 20% of patients in the combination with Peg-IFNα and in 57.1% in the combination with TDF.

Perspectives

LNF, Myr and REP 2139 dysplay distinct activity against the HDV; however, treatment optimization requires the use of Peg-IFN, which remains the backbone of therapy.

Given up to 48 weeks, the virologic response to Myr and LNF in combination with Peg-IFN or with TDF was transient; the invariable virus rebound requires therefore that these therapies be protracted long-term. However, whether HDV can ultimately be eradicated by extending therapy and how long should and could the patients be treated to achieve a durable response remains unknown.

In all studies the cardinal criterion of efficacy was the clearance of HDV RNA from serum. However, a negative viremia is not a dependable end-point of therapy in CHD; several studies have shown that the only robust end-point is the clearance of the HBsAg [23-26]. The paradox is explained by the low sensitivity of the current assays for HDV RNA compared to the infectivity of the virus in a HBsAg background. The sensitivity threshold of current assays for serum HDV RNA is about 10 UI/mL but in transmission studies in HBsAg-positive chimpanzees, the infectivity titer of the HDV reached as many as 10^{-11} dilutions of a HBV/HDV serum [27]. Thus CHD patients who apparently clear HDV RNA by current testing, may still harbour the HDV in undetectable amounts, which can be rescued to a full viral recurrence by the HBsAg in liver; during a follow-up of 4 years, viral relapses occurred in 56% of the CHD patients who obtained a SVR in the Peg-IFN HIDIT-1 trial [5].

As neither Myr nor LNF combinations are capable to clear the HBsAg in the short term and no other hard end-point is at hand, the evaluation of response should be more pragmatic and

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rebalanced to prioritizing the clinical response (normal ALT/AST and improved liver function) rather than the virologic response. In support to this unorthodox approach, a decline of 2 or more logs of HDV RNA without achieving a negative HDV RNA test has been recently proposed as a surrogate marker for treatment efficacy in clinical HDV trials, provided that ALT are normalized [28].

Prolonged treatments raise the problem of safety, in particular in association with the poorly tolerated Peg-IFNα. Peg-IFNλ might provide an alternative, as this cytokine is credited to induce less side effects that Peg-IFNα; however, whether it is more suitable for prolonged treatments in CHD remains to be demonstrated.

Long-term studies will hopefully determine whether the new therapies can further increase eradication of HDV within a reasonable time of treatment compatible with the tolerance and safety of the patient, and whether they may be adjusted to maintain latent, clinically inactive HDV infections with continued therapy.

In the perspective of long-term treatments, Myr monotherapy seems to provide a valid alternative. Though less active against the HDV than the combinations, it has driven good biochemical responses and has been generaly well tolerated; elevations of bile salts were not associated with itching and transient neutropaenia, thrombocytopaenia and eosinophilia were all clinically uneventful. Myr monotherapy would seem the only viable option for the many cirrhotic HDV patients who are at risk with Peg-IFNα.

At present Hepatitis D still remains an urgent but unmet medical need. More efficient drugs are required to eradicate the HDV. Efforts are under way to develop new HBV drugs focused on the clearance of the HBsAg through the silencing or degradation of the HBV cccDNA that provides the template to the synthesis of the antigen [29]; by depriving the HDV of its HBsAg substrate, their success could by default be of benefit to CHD.

More important, the pool of antiviral drugs is enlarging following the recent change of paradigm in antiviral developments, from a virus-centered to host-oriented targets through the repositioning of drugs [30].

There is already a further potential return in CHD. A recent study has shown that the pyrimidine biosynthesis enzyme CAD is a key host factor for HDV infection and an antiviral target in human hepatocytes [31]. CAD is regulated by activated estrogen receptor alpha (ESR1) and in primary human hepatocytes the ESR1 antagonist Fulvestrant exhibited a marked and dose-dependent antiviral effect against HDV through the down-regulation of CAD; the decrease of HDV RNA presumably occured through uridine starvation. Fulvestrant may thus represent the first of a class of agents driving host interactions to target the replication process of the HDV, i. e. the most vulnerable step in its life-cycle.

Conflict of interest

Nothing to disclose.

Funding

No external funding was received.

Authors' contribution

Writing – Original Draft: G.P.C. and M.R.; Writing – Review & Editing: M.R.; Investigation: G.P.C. and M.R.

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Fig. 1. HDV life cycle and targets of the new therapeutic strategies. The Sodium Taurocholate Cotransporting Polypeptide (NTCP) is a functional membrane receptor for the HDV; by docking to the NTCP, Myrcludex B blocks the entry of the HDV into hepatocytes. Farnesylation of the large HD antigen is required to combine the HDV ribonucleoprotein with the HBsAg. By interfering with the farnesylation of the large HD-antigen, Lonafarnib inhibits virion assembly. By blocking the discharge of the HBsAg, REP 2139 prevents the release of the HD virion.