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EDITORIAL

Molecular and clinical aspects of hepatitis D virus infections

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Abstract

Hepatitis D virus (HDV) is a defective virus with circular, single-stranded genomic RNA which needs hepatitis B virus (HBV) as a helper virus for virion assembly and infectivity. HDV virions are composed of a circular shape HDV RNA and two types of viral proteins, small and large HDAgs, surrounded by HBV surface antigen (HBsAg). The RNA polymerase Ⅱ from infected hepatocytes is responsible for synthesizing RNAs with positive and negative polarities for HDV, as the virus does not code any enzyme to replicate its genome. HDV occurs as co-infection or super-infection in up to 5% of HBsAg carriers. A recent multi-center study highlighted that pegylated interferon α-2a (PEG-IFN) is currently the only treatment option for delta hepatitis. Nucleotide/nucleoside analogues, which are effective against HBV, have no relevant effects on HDV. However, additional clinical trials combining PEG-IFN and tenofovir are currently ongoing. The molecular interactions between HDV and HBV are incompletely understood. Despite fluctuating patterns of HBV viral load in the presence of HDV in patients, several observations indicate that HDV has suppressive effects on HBV replication, and even in triple infections with HDV, HBV and HCV, replication of both concomitant viruses can be reduced. Additional molecular virology studies are warranted to clarify how HDV interacts with the helper virus and which key cellular pathways are used by both viruses. Further clinical trials are underway to optimize treatment strategies for delta hepatitis.

Key words: Hepatitis D virus; Hepatitis B virus; Delta hepatitis; Liver cirrhosis; Hepatocellular carcinoma; Hepatitis B virus surface antigen

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INTRODUCTION

The hepatitis D virus (HDV) causes chronic or fulminant hepatitis in patients, as a co- or super-infection in hepatitis B virus (HBV) infected individuals[1,2]. Experimentally, the "delta virus" can also infect chimpanzees or woodchucks who have already been infected with HBV or woodchuck hepatitis virus (WHV), respectively[3]. HBV/HDV co-infection is a clinically very deleterious condition as it commonly leads to progression of hepatic fibrosis, cirrhosis and increase the risk of hepatocellular carcinoma[4]. The mechanism by which HDV promotes hepatic malignancies are unclear, however it has been shown that the virus has negative effects on hepatocytes' growth and viability[5].

HDV, with a spherical shape and a virion size of 36 nm, is a satellite virus with a circular RNA of negative polarity which requires obligatory a helper function to propagate[1,2]. This helper function is usually provided through HBV by sharing its envelope proteins[2]. Some *in-vivo* studies have shown that other *Orthohepadnaviri-*

dae members like WHV can also play the same role as HBV[6,7]. There are some arguments that interactions between HDV and its helper virus might not be limited to surface protein supplies, but also other unknown mechanisms[2]. With respect to the size of the genome (1679 bp RNA), the rolling circle mechanism of replication and its high GC nucleotide content, HDV is very unique among animal viruses and looks very similar to viroids (a family of helper-independent plant pathogens)[8].

VIRAL STRUCTURE

The HDV ribonucleoprotein (RNP) is surrounded by a mixture of host cell-derived lipids and HBV surface proteins (HBsAg)[9]. HDV has a small single stranded RNA genome of 1679 nucleotids[10]. Due to the high degree of intramolecular base pairing in the HDV genome, the RNA folds to an un-branched, rod like structure[11]. HDV's circular RNA forms a complex with two viral-encoded proteins, small (195 amino acids) and large (214 amino acids) HDV-antigens (HDAg-S or p24, and HDAg-L or p27, respectively)[6,9,11]. Delta proteins have identical amino acids except for 19 additional residues at the C-terminus of HDAg-L[9].

The small delta protein acts as a trans-activator for initiation of genomic RNA replication[12]. It also undergoes post translational modifications like methylation, acetylation and phosphorylation to mediate viral mRNA transcription in its modified form[10,13]. HDAg-L is a late protein which inhibits viral RNA replication and transcription of viral proteins, interacts with HBV surface antigens and accelerates the assembly of new virions[6,9,11]. The capacity of binding to HBsAg is not only limited to HDAg-L protein but also occurs with HDAg-S. In fact, in the presence of HDAg-S the packaging level of HDV raises up to 3 to 4 fold[9]. Moreover, there is a conserved molar ratio of delta antigen to HDV genomic RNA in infected liver tissues as well as in HDV particles[10].

MOLECULAR INTERACTIONS BETWEEN HBV AND HDV

HBV, with a 3.2 kb partial double stranded DNA, plays the role of a HDV helper virus in HBV/HDV-infected hepatocytes. From the four overlapping reading frames in the HBV genome one encodes viral surface envelope proteins[14]. This region contains (from amino- to carboxyl-end) the pre-S1 (119 aa), pre-S2 (55 aa) and S (226 aa) domains[9]. These domains encode the large (from pre-S1, pre-S2 and S), middle (from pre-S2 and S) and small (from S) HBV surface proteins which are all in the same translational frame with different start codons (N-terminals)[6,9].

Although the S-HBsAg alone is sufficient for virion development due to its self-assembling trait (which most of the time leads to empty envelope particle formation), the presence of large HBsAg is necessary for both HBV and HDV to infect other cells[6,9]. *In-vivo* studies have shown that all three HBsAg proteins are present in HDV particles[9]. Delta proteins can bind to the S domain of HBV envelope as well as the L4 region which is located in the pre-S1 domain of HBsAg (amino acid residues 86-108). This is the same region which HBV core proteins interact with[9].

The surface protein coding region of HBV overlaps with polymerase encoding genes of HBV, so any mutation in this region may affect both polymerase and HBsAg activities[6]. In fact, mutations in the HBV polymerase gene that also affect correspondingly the HBV surface antigen coding region, may therefore have effects on HBV as well as on HDV replication efficacy. Not all consequences of HBV mutations for HBV/HDV viral replication are currently understood, but limited data exist for some of the most common clinical variants (Table 1). Effects of mutations within the HBV polymerase and surface proteins on HDV secretion. LMV stands for Lamivudine. Data are mainly derived from molecular interaction studies *in vitro*[6,15,16]. While the lamivudine-resistant mutant rtM204V improves HDV secretion, another resistance-conferring mutant like rtM204I (corresponds to sW196L/S/stop) diminishes HDV production[6], which is due to the importance of the codon 196 in the HBV surface antigen for HDV packaging[6,15].

Mutations in C-terminal region of the envelope proteins (especially between amino acid residues 163 to 224) can severely reduce HDV assembly[16]. HBsAg mutations, selected by antiviral agents, are also located in this region[6]. These mutations can affect the level of HDV virion secretion out of the cells in *in-vitro* experiments[6,16].

Although it is known that HBV/HDV-coinfected patients have an unfavorable clinical outcome, the exact role of HBV and HDV in liver disease progression has been controversially discussed, because contrasting results have been published about the role of each one of the viruses[10,12]. Some authors suggested that the underlying HBV infection is aggravated by concomitant presence of HDV in hepatocytes with synergistic deleterious effects on cell survival[12,17], while others indicated that HDV, which often suppresses HBV, is most critical for liver disease and malignant transformation[5,10,12]. From a molecular point of view, it is very likely that both viruses do not replicate independent from one another, but modulate each other's replication level and also pathogenicity[2]. Suppressed HBV replication, but also high or fluctuating loads of both viruses in the serum of co-infected individuals have been reported[2,12]. A recent longitudinal study investigating quantitatively HBV and HDV viral loads in co-infected patients showed that in spite of cross-sectional studies, in which there is a significant positive association between HBsAg level and HBV-DNA or HDV-RNA, HBsAg has longitudinally fluctuating levels in the presence of HDV[12]. Apparently, there is not always an inhibitory effect of HDV on HBV replication as sometimes HBV shows significant inhibitory actions on HDV in HBV/HDV double infection as well.

Table 1 Molecular interactions between hepatitis B virus and hepatitis D virus

HBV polymerase mutation(s)	Corresponding HBsAg mutation(s)	Clinical relevance	Effect(s) on HDV
rtM204V	sI195M(A)	LMV resistance	Enhanced HDV secretion
rtM204I	sW196L/S/stop	LMV resistance	S, L: no HDV secretion
rtD205H	sW196F	Selected during LMV treatment with reduced binding to anti-HBsAg antibodies	Reduced HDV secretion
rtV173L	sE164D	Selected during LMV treatment with reduced binding to anti-HBsAg antibodies	Reduced HDV secretion
rtV173L/rtM204V	sE164D/sI195M	Selected during LMV treatment with reduced binding to anti-HBsAg antibodies	Support HDV secretion

LMV: Lamivudine; HDV: hepatitis D virus; HBV hepatitis B virus; HBsAg: HBV surface antigen.

This observation might be due to some changes in HBV surface antigen region interacting with HDV[12]. Overall, the molecular mechanisms underlying HBV/HDV interactions leading to the progression of the disease are still uncertain and require intense further investigations.

PROTEIN MODIFICATIONS OF HDV

The level of HDV replication and pathogenicity is not only influenced by interactions with HBV and HBV mutants, but might be also impacted by modifications of the HDV proteins as well. As such, some amino acid residues in S- and L-HDAg appear to be critical for posttranslational modifications[1,10]. Of these residues Arg-13, Lys-72 and Ser-177 in S-HDAg undergo methylation[18], acetylation[19] and phosphorylation[20], respectively[1,10].

There are also multiple lysin residues throughout the whole S-HDAg as well as 66 amino acids at the N-terminal part of this protein which act as sumoylation sites of the protein. Sumoylation is a reversible process which has implications for cell cycle progression, nuclear import, regulation of transcription, protein turnover and other cell biology functions. In case of HDV, sumoylation enhances G-RNA and mRNA synthesis by unknown mechanism but has no effect on antigenomic RNA (AG-RNA) synthesis[1].

In L-HDAg Cys-211 gets isoprenylated for virus assembly[1,21,22]. Deletion of 15 amino acids upstream of the isoprenylation site would also lead to the eradication of viral replication[21]. Moreover, some mutants of HDV have been observed which can only replicate in the presence of wild-type HDV, called "defective" viruses[23].

HDV REPLICATION

The replication cycle of HDV is schematically summarized in Figure 1. N-terminal residues of Large Hepatitis B surface antigen mediate the entry of HDV into the hepatocytes[24]. Once delta virus enters the cell it gets uncoated, and the accompanying S-HDAg leads the HDV nucleoprotein complex to the cell's nucleous[10,11]. HDV has a mechanism of double rolling circle amplification. For this, the virus needs an RNA-dependent RNA polymerase activity which in the majority of RNA viruses, but not in the case of HDV, is carried out by virally encoded enzymes[25]. Exceptionally, the delta hepatitis virus is capable of using host RNA polymerases to amplify its genome[25].

The HDV genomic strand undergoes RNA-dependent RNA synthesis, more likely by nucleolar RNA-POL-I[1,11], to produce multimeric full-length intermediate RNAs or AG-RNAs. These molecules then serve as templates for cellular RNA Pol-Ⅱ to generate HDV genomic RNA again through another rolling circle step[11]. Both genomic and anti-genomic strands of HDV contain 85 nucleotides with ribozyme activity which enables the virus to self-cleavage and to ligate its circular RNA[2]. There is also the possibility of producing small segments of RNA transcripts, from both genomic and anti-genomic HDV RNAs which have been consumed to contribute to viral replication[25].

Genomic RNA is also transcribed into an mRNA (0.8 kb) which encodes the HDAgs[1]. This step distinguishes HDV from viroids since they do not produce any protein. This event has also been shown to take place in the nucleus, the same place at which G-RNA synthesis happens[1]. It means that different cellular machineries are mediating HDV genomic RNA/mRNA and HDV anti-genomic RNA synthesis, which are localized in the nucleus and nucleolus of the host cells, respectively[1,11,26,27]. Modified small HDAg intermediates viral mRNA transcription[10,13].

During small HDAg production, an RNA editing event happens at position 1012 by double-stranded RNA-specific adenosine deaminase[22], resulting in alteration of the stop codon of the HDAg-S open reading frame (ORF) and translation extension for additional 19 amino acids[11]. This edition is very essential for the virus since it creates an ORF for the large delta antigen to be translated[22]. The extra 19 amino acid sequence of p27 is poorly conserved among different HDV isolates. However a CXXX motif inside this region causes prenylation of the protein, facilitates protein-protein interactions and directs it to the host cell membrane[28]. Defective mutants of this motif are not able to interact with HBsAgs and to be packaged[28,29]. Expression of L-HDAg initiates interactions with HBV surface proteins and HDV RNP encapsidation[9,11].

TREATMENT OF DELTA HEPATITIS

Chronic hepatitis D, "delta hepatitis", principally demands effective therapy, due to the adverse natural history of chronic HDV infection with more severe liver disease, rapid progression to cirrhosis, increased hepatic decompensation and higher mortality rates compared to HBV

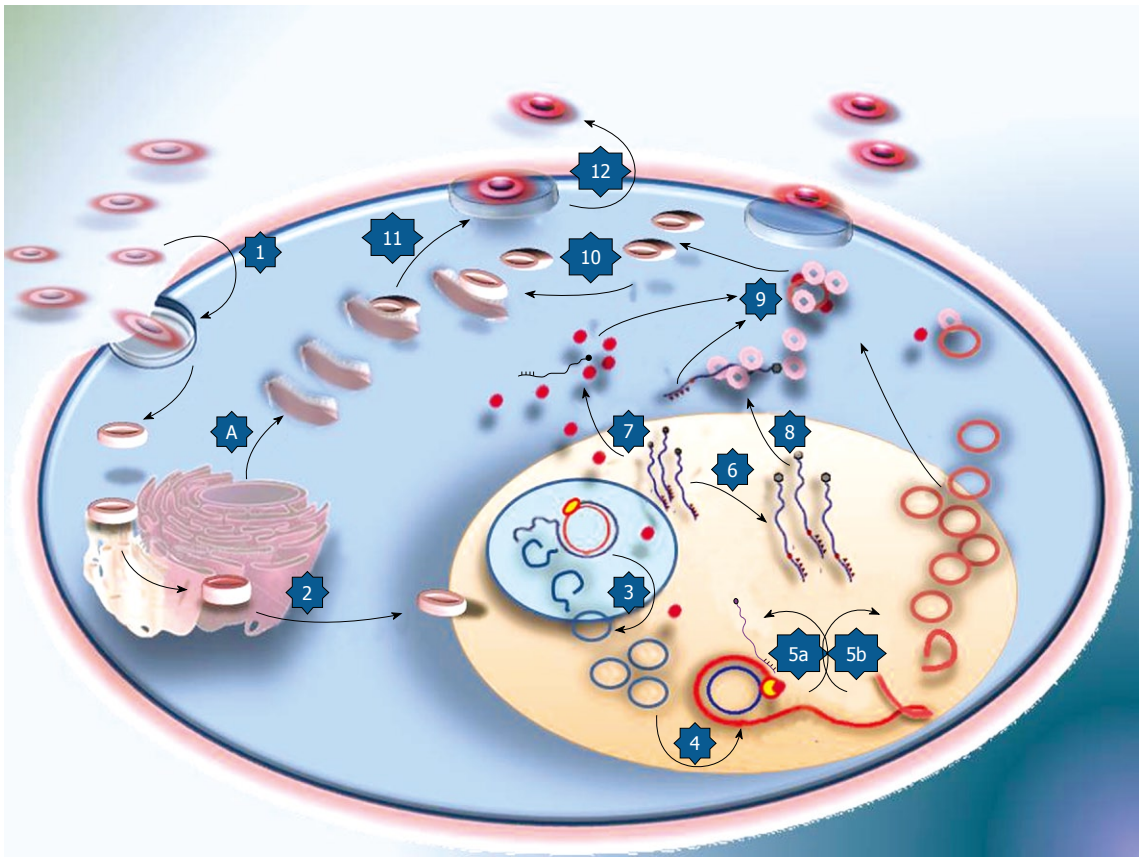

Figure 1 Hepatitis D virus life cycle in hepatocytes in the presence of hepatitis B virus. Schematic summary of the current concept of hepatitis D virus (HDV) replication cycle. The entry of HDV particles into hepatocytes is mediated by the attachment of hepatitis B virus (HBV) surface antigens coating HDV nucleoprotein to the host cell receptors, followed by endocytosis and uncoating of the virions (1). HDV nucleoprotein complex is leaded to the nucleus by accompanying S-HDAg, then the complex arrives to the nucleolus where RNA Pol Ⅰ exists (2). Multimeric full-length antigenomic RNAs (AG-RNAs) are transcribed from HDV genomic RNA, likely by RNA Pol Ⅰ. Circular AG-RNA molecules which are created by ribozyme activities of AG-RNA itself move to the nucleoplasm (3). AG-Strands serve as templates for RNA Pol Ⅱ (4). RNA Pol Ⅱ starts generating mRNA from genomic strands (5a) and full-length transcripts from AG-strands (5b). 6: Due to RNA editing at position 1012 of S-HDAg exerted by double-stranded RNA-specific adenosine deaminase, the open reading frame of these mRNA molecules extends for additional 19 amino acids, which lead to the production of Large delta antigen (6). mRNA molecules coding for small and large delta antigens move to the cytoplasm and are translated to relevant proteins (7,8). Small delta antigens activate genomic RNA replication (7), while large proteins promote virion assembly (8). Viral proteins form nucleoprotein complexes with HDV genomic RNAs (9). L-HDAg in HDV ribonucleoprotein complex interacts with existing HBV surface proteins in the cell (10). After HDV encapsidation by HBV surface proteins (11), complete virions leave the cell through exocytosis (12). HBV surface proteins bud through the endoplasmic reticulum or golgi body membranes of the host cell (A).

monoinfection[30,31]. However, the therapy of chronic hepatitis D infection is a major challenge, because there is no specific virus inhibitor. At the moment, there is only one approved therapy with IFN α or PEG-IFN α, respectively, available[30]. A lot of different substances have been investigated; Table 2 gives an overview of the results of selected major clinical trials.

The recent standard therapy for delta hepatitis is PEG-IFN, administered 180 μg s.c. once per week for a period of 48 wk. Traditionally, conventionally IFN proofed to be effective in chronic hepatitis D in the early 1990s[32-35]. Also, a placebo-controlled trial reported in 2005 could show a benefit for conventional IFN in a small cohort[36]. But nevertheless response rates in matters of sustained virological responses (SVRs) are low and therapy efficacy seems to be proportional to the dose and duration of treatment[37]. In a single case with a 12-year permanent therapy with 5 million units IFN daily a HDV RNA clearance could be achieved after several years accompanied with anti-HBs seroconversion. This was attended by improvement of liver histology (initially cirrhosis, after 10 years no abnormalities)[38]. Interestingly Yurdaydin *et al*[39] could not find a benefit for IFN treatment over 2 years instead of 1 year. However long-term data (12 years) showed a benefit of high-dose IFN α therapy (9 million units three times per week) with even regression of advanced hepatic fibrosis[40].

Due to the advantageous pharmacodynamics and pharmacokinetics, PEG-IFN clearly provided a benefit in HDV therapy. In 2006 three small studies could show effectiveness for this type of treatment[41-43], but nevertheless SVR rates remained low (17% to 43%) overall. In a multicentre randomised landmark trial published in 2011, Wedemeyer *et al*[44] achieved 28% SVR. These differences in SVR rates compared to prior smaller trials might be due to baseline clinical, demographical and virological characteristics[30]. Farci[37] proposed to divide patients into IFN responders and non-responders. Additionally,

Table 2 Selected clinical trials on delta hepatitis therapy

Ref.	Drug used	Dosage	No. of patients included	Main result (s)
Garripoli *et al*[45], 1994	Ribavirin monotherapy	15 mg/kg for 16 wk	9	Ribavirin did not show significant antiviral effects in chronic hepatitis D
Wolters *et al*[49], 2000	LAM + IFN add-on	LAM 100 mg at least for 24 wk; afterwards combination therapy with IFN 9 MU/d for 4 wk, followed by 9 MU 3 times/wk for 12 wk	8	Neither LAM alone nor the addition of IFN was capable of reducing HDV
Yurdaydin *et al*[47], 2002	Famciclovir	500 mg for 6 mo	15	Not effective
Farci *et al*[40], 2004	High-dose IFN α *vs* low dose IFN *vs* no treatment	High dose: 9 million units 3 times/wk, low dose 3 million units 3 times/wk for 48 wk	36	High-dose IFN α significantly improves long term clinical outcome and survival
Kaymakoglu *et al*[46], 2005	IFN α + ribavirin	IFN 10 MU 3 times/wk, Ribavirin 1000-1200 mg/d for 24 mo	19	Addition of Ribavirin to IFN-α does not increase response rate in patients with CHD
Niro *et al*[36], 2005	LAM *vs* placebo	100 mg LAM for 52 wk	31	HDV viraemia was unaffected, even in patients when HBV replication was lowered by LAM therapy
Erhardt *et al*[43], 2006	PEG-IFN	1.5 μg/kg PEG-IFN per wk for 48 wk	12	PEG-IFN is a promising treatment option in chronic hepatitis D
Castelnau *et al*[41], 2006	PEG-IFN	1.5 μg/kg PEG-IFN per wk for 12 mo	14	PEG-IFN is safe and efficient for HDV treatment
Niro *et al*[42], 2006	PEG-IFN mono *vs* combination therapy with ribavirin	1.5 μg/kg PEG-IFN per wk; 800 mg ribavirin; 48 wk mono or combination therapy, afterwards 24 wk PEG-IFN mono	38	Ribavirin had no effect
Yurdaydin *et al*[50], 2008	LAM *vs* LAM + IFN *vs* IFN mono	IFN 9MU 3 times/wk, LAM 100 mg; totally 12 mo therapy; for combination therapy 2 mo LAM mono, afterwards 10 mo combination	39	Addition of LAM to IFN is of no additional value; both (IFN mono/IFN + LAM) are superior to LAM mono
Mansour *et al*[52], 2010	PEG-IFN, add-on tenofovir und emtricitabine after 2 mo	PEG-IFN 180 μg/wk; tenofovir 300 mg/d for 10 mo	1	Combination therapy with PEG-IFN and nucleoside/tide analogue seems to be more effective than IFN alone
Wedemeyer *et al*[44], 2011	PEG-IFN mono *vs* adefovir *vs* combination PEG-IFN + adefovir	PEG-IFN 180 μg/wk; adefovir 10 mg/d for 48 wk	90	PEG-IFN α-2a with or without adefovir resulted in sustained HDV clearance in about 25%

LMV: Lamivudine; LAM: Lamivudine; CHD: Coronary heart disease; HDV: hepatitis D virus; HBV hepatitis B virus; PEG-IFN: Pegylated interferon.

IFN responders might be distinguished in early- and late-responders. The latter could possibly benefit from a prolonged treatment, because HDV decrease might occur late, even after the end of treatment[44]. Clear predictors of response to IFN have not been identified, but viral load determination at 6 mo of treatment might be helpful[41,43]. Even in virological non-responders, Erhardt *et al*[43] observed a stabilization of histological liver score under therapy so that IFN might be beneficial.

Because of the poor results and high rates of adverse events like flu-like symptoms or bone marrow suppression with anemia or neutropenia, dose reduction or discontinuation of treatment are common upon PEG-IFN therapy, corroborating the urgent need for therapeutic alternatives.

Major problems in developing new treatment strategies are that there are no specific therapeutic targets like a virus polymerase and that potentially two viruses have to be treated at the same time[30]. A lot of different approaches have been investigated over the last years. Ribavirin as a monotherapy or in combination with IFN could not show a benefit[42,45,46], likewise Famciclovir[47] and acyclovir[48] had no effect. Different studies could not show an advantage of lamivudine as a monotherapy or combination therapy[36,49,50]. Because of a significant decrease in cccDNA levels accompanied with a reduction in serum HBsAg titers in long-term adefovir therapy[51], the well-known HBV-effective nucleotide analogue adefovir dipivoxil was thought to be a potential anti-HDV drug as well. Interestingly, in the recent HIDIT-1 study Wedemeyer *et al*[44] could not find superiority compared to PEG-IFN monotherapy. Thus, nucleoside/nucleotide analogue treatment is not recommended at the moment in patients with suppressed or low HBV replication. In a single case report, a patient achieved a SVR accompanied with anti-HBs seroconversion after add-on therapy of tenofovir and emtricitabine to PEG-IFN[52]. Therefore, further studies are required to investigate the role of nucleoside/nucleotide analogues with high resistance barrier like entecavir or tenofovir. The HIDIT-2 trial, for instance, combines PEG-IFN with tenofovir for the treatment of HDV, and results from this trial are anticipated within the next years.

Because of different patterns in replication of HDV and HBV (active HDV/inactive HBV 70%, active HDV and HBV 23%, inactive HDV/active HBV 4%, both inactive 3%)[53] which vary over time a close treatment surveillance and an individually adopted therapy is likely to be essential. Possibly, patients with a high HBV replication might benefit from a therapy with nucleoside/nucleotide

analogues, because of the long-term HBsAg reduction observed with these agents. Sheldon *et al*[54] could show that in a long-term study (median 6.1 years) of HIV/HDV/HBV co-infected patients who where treated with anti-HBV drugs 13 of 16 patients had reduced HDV viremia and ALT levels. Even three of these achieved undetectable HDV RNA and normal ALT levels. This is especially important considering that high levels of HBV-DNA in HDV/HBV co-infected patients lead to more severe liver damage than those with low viremia[55].

Another promising approach is the use of prenylation inhibitors. Bordier *et al*[56] used a farnesyltransferase inhibitor (FTI) because prenylation of the large delta antigen - especially the prenyl lipid farnesyl, which was found on the delta antigen[57] - seems to play an essential role for the virus assembly and release. In this study, a complete clearance of HDV viremia with FTI was achieved[56]. Orally taken FTIs have been developed with a relative lack of toxicity in human phase I / II studies[58] and might be a potential new substance group for treatment of chronic HDV infection.

In a pilot study of chronically infected woodchucks clevudine was capable to reduce WHV cccDNA with reduction in WHsAg. Moreover, they could achieve in 75% of HDV infected woodchucks undetectable HDV RNA with clevudine treatment[59]. An HBsAg titer reduction by reducing the cccDNA during clevudine therapy was also found in humans[60]. In comparison to lamivudine, clevudine seems to be superior in HBeAg positive HBV. Compared to entecavir, in chronic HBV infected patients clevudine could reduce viral load similar than entecavir, but higher rates of virological breakthrough and significantly more myopathy was observed[61], indicating that clevudine has a higher adverse event profile.

Potential novel strategies for an anti-HDV treatment might be an HDV receptor blockade, which is thought to be the same receptor like HBV. Also, a modulation of the balance between S-HDAg and L-HDAg and especially modification of post-translational changes of HDAg, which effects the viral life cycle, might be a promising target. Another approach is the reduction of HBsAg, which might be associated with clearance of HDV RNA. Vietheer *et al*[6] for instance could show that mutations in the HBsAg lead to an inhibition of HDV particle secretion. But it should be taken into consideration that HDV, once it got into the cell, can replicate without HBV[62-64], so that an HBsAg reduction might inhibit new infections of cells, but can theoretically not by itself promote the clearance from already infected cells. Also other IFN types, like IFN λ[30], should be evaluated for their efficacy in HDV.

At the moment, the current standard therapy for chronic HDV infection is PEG-IFN α-2a 180 μg s.c. weekly. Wedemeyer *et al*[44] proposed a treatment for 48 wk. If there is a high viremia with positive anti-HDV IgM after this treatment period, a response seems to be unlikely. If there is a reduced viremia, decreased IgM antibody titers or transaminases patients might benefit from extended therapy for 72 wk. Patients with a high HBV replication might benefit by nucleoside/nucleotide therapy. Also a spontaneously HDV clearance might appear with spontaneously seroconversion to anti-HBs (0.25% annual rate). Nevertheless, therapy is needed in delta hepatitis, because HDV replication is an independent predictor of mortality[65] and lack of treatment is a predictor of an unfavourable outcome[53]. Even 8/35 patients with an SVR developed a HCC in long-term[65], especially elderly are at a specific risk[66].

The aim of therapy is a HDV RNA clearance, seroconversion to anti-HBs and avoiding imminent complications like cirrhosis. Once a stage of liver cirrhosis has developed, the viral clearance will have limited influence on the further course of liver disease. Half of patients who develop cirrhosis later will progress to liver failure[53]. To cure patients, long-term IFN treatment is required for undetectable HDV RNA and further treatment is required for HBsAg loss[67]. During therapy, monitoring of HDV RNA and HBsAg might help in the surveillance of therapy, although HDV RNA does not correlate with activity or stage of liver disease[68].

Overall, more long-term data as well as a better understanding of the viral life cycle and HDV/HBV interactions are needed for an efficient HDV treatment. Until then, HDV infection obviation should be a major focus of health care measures by preventing delta hepatitis using vaccination against HBV, especially in countries with high HDV prevalence[31].

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