Natural Course and Treatment of Hepatitis D Virus Infection

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Hepatitis D virus (HDV) is a subviral satellite with hepatitis B virus (HBV) as its natural helper virus. After entry into hepatocytes, it utilizes host cellular enzymes to replicate by a double-rolling-circle mechanism. HDV is most often transmitted by contact with contaminated blood and body fluid, similar to HBV infection. Approximately 5% of the global HBV carriers are coinfected with HDV, leading to a total of 10–15 million HDV carriers worldwide. HDV infection can occur concurrently with HBV infection (coinfection) or in a patient with established HBV infection (superinfection). The pathogenesis of HDV remains controversial. A decline in the prevalence of both acute and chronic hepatitis D (CHD) has been observed worldwide. At present, therapy for chronic HDV infection is by the use of interferon-α. Compared to chronic hepatitis B or C, CHD treatment requires a higher dosage and a longer duration of treatment, and post-treatment relapses are common. In order to prevent the progression of CHD and its related morbidity and mortality, more effective treatments are needed. [J Formos Med Assoc 2006;105(11):869–881]

Key Words: genotype, hepatitis B virus, hepatitis D virus, seroepidemiology, treatment

In 1977, Rizzetto and coworkers discovered the hepatitis D virus (HDV) while they were trying to find out why some patients with hepatitis B virus (HBV) infection had more serious disease than others. A previously unrecognized antigen in the liver cell nuclei of patients with hepatitis B surface antigen (HBsAg)-positive chronic liver disease was demonstrated, which they provisionally called the delta antigen.1 Subsequent experiments revealed that the delta antigen was a novel infectious agent we now know as HDV.2 Classified within the new genus Deltavirus,3 HDV is a subviral satellite with HBV as its natural helper virus.2 Cloned and sequenced in 1986, HDV is the first and only animal virus to have a circular, single strand minus RNA genome, which is a structural characteristic and mode of replication that has only previously been seen in plant viroids and virusoids.4–6

HDV Biology

Viral structure
On electron microscopy, HDV is an enveloped, spherical, approximately 36 nm particle that contains a RNA genome of about 1700 nucleotides, the smallest animal viral genome.1,2 The HDV genome has the ability to fold into a partially double-stranded, unbranched, rod-shaped structure, reflecting the high degree of intramolecular base-pairing due to high G+C content.4 The HDV virions possess an outer envelope composed of HBsAg proteins and host lipids, and an inner nucleocapsid consisting of viral RNA and hepatitis delta antigen (HDAg). HDAg, the only HDV-encoded protein discovered so far, consists of two protein species: a 27-kDa (214 amino acids) large HDAg (L-HDAg) and a 24-kDa (195 amino

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acids) small HDAg (S-HDAg), both from a single coding open reading frame (ORF). The two HDAGs are identical in sequence, except that the L-HDAg contains an additional 19 amino acids at the C-terminus.

**HDV viral life cycle**

The replication cycle of HDV starts with the attachment of the virus to the hepatocyte membrane and ends with its release from the infected cell. It is believed that HBsAg-mediated binding to a cellular receptor helps HDV penetration into the hepatocyte. Nuclear localization signal domain on HDAg further leads its genome into the nucleus, where genome replication occurs.

Replication of the RNA is proposed to occur by a double-rolling-circle mechanism, similar to that proposed for the replication of plant viroids and small satellite RNAs of some plant viruses. During replication, three major species of RNA exist in infected hepatocytes: genomic RNA (negative polarity), antigenomic RNA (positive polarity), and a smaller polyadenylated RNA (0.8 kb) of antigenomic polarity, which is the messenger RNA (mRNA) containing the ORF for the synthesis of HDAg. HDV has the unique ability to utilize host RNA-dependent RNA polymerase to transcribe the viral RNA genome directly without a DNA intermediate. At first, the genomic RNA serves as a template to synthesize multimeric linear transcripts of antigenomic polarity, which undergo autocatalytic cleavage and ligation to produce circular monomeric antigenomic RNA. The antigenomic RNA serves as the template for production of more genomic RNA, which is processed to produce the polyadenylated mRNA. S-HDAg, the product of the unedited transcript, stabilizes HDV RNA circle and promotes replication. The antigenomic RNA can also be edited by intracellular host dsRNA adenine deaminase, resulting in a protein with an additional 19 amino acids at the C-terminus, the L-HDAg. The L-HDAg functions as a potent dominant negative inhibitor of viral replication.

Recent studies have shown that post-translational modifications of HDAg can modulate HDV RNA replication. So far, the known post-translational modifications of HDAg include prenylation, phosphorylation, acetylation and methylation. Prenylation of the cysteine residue within the C-terminus of L-HDAg creates a lipophilic molecule that binds to both HDV RNA and to HBsAg, leading to membrane association, viral packaging and release. The phosphorylation of HDAg occurs at multiple residues, with serine-177 (S-177) being the major site. S-177 phosphorylation is required for efficient viral replication, particularly the antigenomic RNA strand. The acetylation of HDAg may regulate the subcellular localization of HDAg and participate in viral RNA nucleocytoplasmic shuttling and replication. The methylation of S-HDAg is also essential for HDV RNA replication, especially for that of antigenomic RNA strand.

Except for its intrinsic autocatalytic activity of genome cleavage and ligation, HDV utilizes and redirects host cellular enzymes toward production of its viral antigen and virions. The study of the viral lifecycle can point to new targets for effective antiviral therapy, one example of which is the development of anti-HDV drugs by inhibiting HDAg isoprenylation.

**Seroepidemiology**

HBV infection is a global health problem, and over 350 million people around the world are chronic carriers of the virus. It has been estimated that approximately 5% of the global HBsAg carriers are also coinfected with HDV, leading to a total of 10–15 million HDV carriers worldwide. A recent seroepidemiologic study in Taiwan revealed that 5% of female prostitutes, 14% of intravenous drug users and 16% of male prostitutes are anti-HDV-positive. In Italy, 8.3% of HBsAg-positive patients in 14 liver referral units were positive for anti-HDV. Chronic HDV infection is estimated to be responsible for more than 1000 deaths each year in the United States. High risk groups include HBsAg-positive parenteral drug users.
users, female prostitutes and brothel-goers.\textsuperscript{26,28} Male prostitutes and immigrant prostitutes have been identified as new “high-risk” populations and may become a reservoir for disease transmission in HBV endemic areas.\textsuperscript{25} Overall, HDV infection is still an important public health problem worldwide and remains a major cause of mortality and liver transplantation.

**Geographic Distribution of HDV Infection**

The geographic distribution of HDV infection might be expected to mirror that of HBV. Nevertheless, the rate of HDV infection is not a simple reflection of that of HBV infection.\textsuperscript{29} There are areas with high prevalence of HBV infection but relatively low prevalence, if any, of HDV infection, suggesting that other factors, such as age at HBV infection, may determine the acquisition of HDV infection. For example, in Alaskan Natives, in which HBV infection occurs during infancy and childhood, the prevalence of HDV infection is negligible despite HBV endemicity. HDV appears to be endemic in the Middle East, but again its distribution bears little relationship to that of HBV.\textsuperscript{30} High prevalence areas of HDV infection include Italy, some parts of Eastern Europe, the Amazon basin, Venezuela, Columbia, some Pacific Islands, Pakistan, and Western Asia.\textsuperscript{29,31–34} It is estimated that each year, 7500 HDV infections occur in the United States.\textsuperscript{32} New foci of infection have been identified in the island of Okinawa in Japan,\textsuperscript{35} villages in China, northern India,\textsuperscript{36} and southern Albania.\textsuperscript{37} The subtropical area in southern America remains an important potential reservoir for new outbreaks of HDV infection.\textsuperscript{38} 

**Viral Heterogeneity**

Longitudinal analysis of the evolution of HDV RNA quasispecies during chronic infection revealed that the mutation rate of HDV isolates ranges from $3 \times 10^{-2}$ to $3 \times 10^{3}$ base substitutions per nucleotide per year.\textsuperscript{39} Highly conserved domains have been identified in the regions around the autocatalytic cleavage site of the genomic and antigenic RNA and the RNA-binding domain of HDAg.\textsuperscript{40,41} Full-length RNA sequence comparisons show up to 40% heterogeneity in genomic sequence variability among isolates collected worldwide,\textsuperscript{42} and at least three genotypes have been identified. HDV genotype may be determined by restriction fragment length polymorphism analysis of polymerase chain reaction (PCR) products, by direct sequencing, and by immunohistochemical staining using genotype-specific anti-HDV antibodies on liver biopsies.\textsuperscript{43}

Being the most common genotype worldwide, genotype 1 is associated with a broad spectrum of pathogenicity.\textsuperscript{44} A recent study further revealed that genotype 1 HDV and genotype C HBV infections were associated with the development of cirrhosis, hepatocellular carcinoma (HCC), or mortality due to hepatic failure in chronic hepatitis D (CHD) patients.\textsuperscript{45} Within genotype 1, two subtypes, 1A and 1B, have been identified. Subtype 1A is predominant in Asia, 1B in the United States, and both are common in the Mediterranean area. Isolates of genotype 2 were originally discovered in Taiwan and Japan, where it is the predominant genotype. An association of genotype 2 with milder forms of liver disease in these areas has been observed,\textsuperscript{46} which may be related to fewer viral particles secretion due to lower packaging and editing efficiency, and hence less severe hepatic inflammation.\textsuperscript{47} Genotype 3 is exclusively found in countries from northern South America (Columbia, Venezuela, Peru). It has been associated with outbreaks of severe and fulminant forms of hepatitis. Interestingly, this genotype is linked to the coexisting HBV genotype F.\textsuperscript{31,48,49} Unlike genotype 3, no particular linkage between HBV and HDV genotypes could be found in Taiwan.\textsuperscript{50} Besides epidemiologic interest, further studies are necessary to clarify whether clinical differences among genotypes exist in terms of efficacy of replication, packaging, infectivity, transmissibility and pathogenicity.

Furthermore, phylogenetic reconstructions based on the HDAg gene and full-length genome
sequence data show an extensive and probably ancient radiation of African lineages, indicating at least seven major clades among HDV isolates. So far, only one serotype of HDV has been identified.

Modes of Transmission

As it is enveloped by HBsAg, HDV has similar transmission routes to those of HBV, with percutaneous exposure being the most efficient one. Intravenous drug use is among the commonest modes of HDV transmission in non-endemic areas, such as in northern Europe and in the United States. In the past, hemophiliacs and polytransfused subjects were at increased risk of acquiring HDV infection, but it has virtually disappeared as a result of universal HBV vaccination and blood screening for HBsAg. Epidemiologic analysis suggests that sexual contact with an HDV carrier is also an important route of HDV transmission. High homology of HDV nucleotide sequence between index patients and their spouses provided additional molecular evidence of a common source of HDV infection in some couples. In HDV endemic areas, such as the Mediterranean basin, the unapparent parenteral route of transmission accounts for most cases of HDV transmission, with a trend to form clusters among family members. In southern Italy, cohabitation with an HDV carrier was identified as a major risk for HDV transmission. Perinatal transmission of HDV is rare.

HDV: A Declining Disease?

A changing trend in the epidemiology of HDV has been observed. For example, a decline in the prevalence of both acute and CHD has been observed in Southern Europe and Southeast Asia. In addition, the occurrence of fresh and severe forms of HDV infection has significantly decreased in Italy. It is postulated that current patients may represent cohorts infected years ago who survived acute HDV infection. In Taiwan, the prevalence of HDV infection among high-risk subjects has also declined over the past two decades. Active preventive measures against promiscuity and sexually transmitted diseases, and the introduction of disposable needles and syringes to eliminate the opportunities for equipment sharing may have contributed to this phenomenon. The estimated rate of decrease among female prostitutes from 1988 to 2002 was 4.2% per year. Male prostitutes and immigrant prostitutes have, in contrast, been identified as the new high-risk populations in Taiwan, and they may become a reservoir for disease transmission. In brief, a decline in the prevalence of HBsAg carriers due to universal HBV vaccination, improvement in socioeconomic conditions, reduced family size, and changes in the behavior of intravenous drug users and in sexual practice in response to HIV infection have probably contributed to the declining incidence of HDV infection worldwide.

Clinical Features and Diagnosis in Different Situations

Acute HDV infection can occur concurrently with HBV infection (coinfection) or in a patient with established HBV infection (superinfection) (Table 1). In patients with HDV coinfection with HBV, a biphasic increase in serum aminotransferase activity has been commonly observed in the clinical setting, which may help to differentiate it from histologically indistinguishable acute HBV infection. However, since no large study has demonstrated the proportion of cases of HDV coinfection presenting with such a clinical manifestation, the accuracy of using this feature to diagnose HBV and HDV coinfection remains unproven. Although the clinical manifestations of HDV and HBV coinfection may range from mild to severe fulminant hepatitis, complete clinical recovery from acute self-limiting disease with no chronic sequelae is usually the rule. The chance of HDV coinfection becoming chronic is intrinsically limited because the chance of the
helper virus HBV infection to become chronic is also uncommon, occurring in less than 10% of adult patients. Due to short-lived HDV, HDV infection has little impact on the natural history of HBV infection in this setting. The rate of chronicity following coinfection with HBV and HDV is equal to that of HBV infection alone.\(^5^9\)

In HBV carriers superinfected with HDV, the pre-existing HBsAg provides an ideal biological substrate for HDV to complete its lifecycle. Clinically, HDV superinfection may be divided into three phases: acute phase, active HDV replication and suppression of HBV with high alanine aminotransferase (ALT) levels; chronic phase, decreasing HDV and reactivating HBV with moderate ALT levels; and late phase, development of cirrhosis and HCC caused by replication of either virus or remission resulting from marked reduction of both viruses.\(^6^0\) In general, in the acute phase, HDV superinfected carriers may develop severe hepatitis, and around 70–90% will progress to chronicity.\(^6^0\) In patients with acute hepatitis A to E, HDV superinfection is also found to be associated with a higher incidence of fulminant hepatic failure.\(^6^0\)–\(^6^2\) An Italian study showed that HDV infection was found in 20 (90%) of the 22 HBV patients who presented with a rapidly progressive course to cirrhosis.\(^6^3\) Furthermore, cirrhosis developed in up to 70% of CHD patients in the superinfection setting, about 15% within 1–2 years of disease onset.\(^6^3\) Liaw et al found that the incidence of cirrhosis development in CHD patients cumulated to 21% in the 5 years following acute HDV superinfection.\(^6^4\) The above evidence indicate that HDV infection plays an important role in the progression of HBV-related liver diseases. From another aspect, a low prevalence of HDV activity was noted in asymptomatic carriers of HBsAg in another Taiwanese study in the superinfection setting.\(^6^4\) In Taiwan, CHD patients with HCC were also not younger than chronic hepatitis B patients with HCC. Acquisition of HDV infection in middle-aged adults through sexual contact with prostitutes may account for the phenomenon in Taiwan, rather than close family contact, or early intravenous drug abuse in Italy.\(^6^5\),\(^6^6\) In brief, HDV superinfection is likely to become chronic simply because the HBV infection is already chronic. Only a few patients have a self-limiting course with subsequent clearance of HBV and HDV. By providing HBsAg for HDV secretion and release, active HBV replication may play an important role by modulating the pathogenetic potential of this defective agent.\(^6^7\) However, the complex interplay between HBV and HDV could explain the

![Table 1. Clinical features of hepatitis D virus (HDV) coinfection and superinfection in hepatitis B virus (HBV) carriers](image-url)

*Using immunoblot assay, detection rate of serum HDAg may be comparable to Northern blot detection of HDV RNA using cDNA probe.*
above controversial findings. Furthermore, persistent HDV infection could also exert effective suppression on HBV replication, with subsequent HBeAg and HBsAg clearance. On the other hand, decreasing level of HDV RNA with reappearance of HBV DNA and progression of liver disease in CHD patients has also been reported in Taiwan. Future studies are required to evaluate the exact role of the individual viruses in the outcomes of patients with dual HBV and HDV infection.

Currently, the most reliable tool to diagnose HDV infection is based on highly sensitive molecular techniques, such as reverse transcription-PCR. In acute HDV infection, serum HDV RNA is detectable in up to 90% of cases during the symptomatic phase of the illness and becomes undetectable after clinical resolution. Diagnosis of HDV infection can also be achieved indirectly based on the detection of antibodies against HDag (anti-HD) of immunoglobulin G (IgG) and IgM classes in serum. Testing for IgM anti-HD is crucial not only as a marker of primary HDV infection but also for its clinical relevance in the natural history of the disease. During follow-up, the decrease in or disappearance of monomeric IgM anti-HD predicts resolution of chronic HDV infection, either spontaneously or induced by interferon (IFN).

In chronic HDV infection, the clinical features are nonspecific. It cannot be easily distinguished from chronic hepatitis of other etiologies based on clinical manifestations alone. The correct diagnosis is suggested by a negative test for IgM anti-HBc and confirmed by the detection of HDV markers. Tests for HDV should be considered to rule out coexistent chronic HDV infection in HBsAg-positive patients with chronic active liver disease, which is best achieved by screening for anti-HDV antibody. CHD is associated with high titers of both IgG and IgM anti-HD, although the IgM are monomeric (7S), not pentameric (19S) as in primary infection. In quiescent chronic HDV infection, most patients have antibodies to HBeAg and a low level of HBV replication, while HBV is suppressed. Serum HDV RNA can be detected in only 60–75% of patients, and is a significant factor associated with elevated ALT levels and liver damage.

Pathogenesis

The pathogenesis of HDV remains controversial. In the acute stage, HDAg or HDV RNA may be directly cytotoxic to hepatocytes, S-HDAg, but not L-HDAg, is directly cytotoxic to the cells when expressed in large quantities. HDV genome replication per se causes a modest inhibition of the rate of cellular growth. In the chronic stage, inflammatory cells surround infected hepatocytes, and various autoantibodies have been detected in the serum of patients with CHD, suggesting the possible involvement of immune responses in HDV pathogenesis. Liver–kidney microsomal antibodies type 3 (LKM-3) against uridine diphosphate glucuronosyltransferases have been particularly implicated in this aspect.

Treatment

Patients with CHD actually carry a much higher risk of developing end-stage liver disease than those with HBV alone. Although new HDV infection has declined in recent years, it is still important to pay attention to this important issue and consider active therapeutic intervention in existing patients with dual chronic hepatitis B and D.

Monotherapy

Interferon

HDV-related chronic hepatitis is difficult to treat (Table 2). IFN is still the only therapy for CHD. The response to IFN varies widely and occurs at different time points, sometimes after discontinuation of IFN as in treating HBV patients. The rate of response is generally proportional to the dose of IFN, with 9 million units (MIU) three times a week being more effective than 3 MIU thrice weekly. At the end of treatment, negativity of HDV RNA and normalization of ALT were 71% and 71%, respectively, in the high-dose
Natural course and treatment of hepatitis D virus infection

<table>
<thead>
<tr>
<th>Reference (yr)</th>
<th>Patient</th>
<th>Regimen</th>
<th>Dosage</th>
<th>Duration (wk)</th>
<th>BR (%) at EOT</th>
<th>VR (%) at EOT</th>
<th>SVR (%) at EOFU</th>
</tr>
</thead>
<tbody>
<tr>
<td>Farci et al83 (1994)</td>
<td>CHD</td>
<td>IFN-α-2α</td>
<td>9 MIU TIW</td>
<td>48</td>
<td>71 (10/14)</td>
<td>71</td>
<td>0</td>
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<tr>
<td></td>
<td></td>
<td>IFN-α-2α</td>
<td>3 MIU TIW</td>
<td>48</td>
<td>29 (4/14)</td>
<td>36 (5/14)</td>
<td>0</td>
</tr>
<tr>
<td>Gaudin et al82 (1995)</td>
<td>CHD</td>
<td>IFN-α-2b</td>
<td>5 MIU/m² TIW, then 3 MIU/m² TIW</td>
<td>16th-16th</td>
<td>66 (7/11)</td>
<td>66</td>
<td>9 (1/11)</td>
</tr>
<tr>
<td>Lau et al83 (1999)</td>
<td>CHD</td>
<td>Lamivudine</td>
<td>100 mg QD</td>
<td>48</td>
<td>0 (0/5)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Wolters et al84 (2000)</td>
<td>CHD</td>
<td>Lamivudine/IFN-α</td>
<td>100 mg QD</td>
<td>40</td>
<td>14 (1/7)</td>
<td>0</td>
<td>0</td>
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<tr>
<td>Yurdaydin et al85 (2002)</td>
<td>CHD</td>
<td>Famciclovir</td>
<td>500 mg TID</td>
<td>24</td>
<td>0</td>
<td>1/15</td>
<td>0</td>
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<td>Rosina et al86 (2002)</td>
<td>CHD</td>
<td>THF-γ2</td>
<td>40 µg QD, then 40 µg BIW</td>
<td>15 days</td>
<td>3rd-24th</td>
<td>0</td>
<td>37 (3/8)</td>
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<td>Niro et al87 (2005)</td>
<td>CHD</td>
<td>Lamivudine</td>
<td>100 mg QD</td>
<td>52</td>
<td>0 (0/25)</td>
<td>8 (2/25)</td>
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<td>Kaymakoglu et al88 (2005)</td>
<td>CHD</td>
<td>Ribavirin/IFN-α</td>
<td>1000–1200 mg QD</td>
<td>96</td>
<td>42 (8/19)</td>
<td>42 (8/19)</td>
<td>21 (4/19)</td>
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<td>Castelnau et al90 (2006)</td>
<td>CHD</td>
<td>PEG-IFN-α-2b</td>
<td>1.5 µg/kg/wk</td>
<td>48</td>
<td>57 (8/14)</td>
<td>43 (6/14)</td>
<td></td>
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<td></td>
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<tr>
<td></td>
<td></td>
<td>PEG-IFN-α-2b/ribavirin</td>
<td>1.5 µg/kg/wk</td>
<td>72/48</td>
<td>41 (9/22)</td>
<td>9 (2/22)</td>
<td>18 (4/22)</td>
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</tbody>
</table>

CHD = chronic hepatitis D; BR = biochemical response, defined by normalization of serum alanine aminotransferase; VR = virologic response, defined by the disappearance of HDV RNA in serum; SVR = sustained virologic response; EOT = end of treatment; EOFU = end of follow-up; THF-γ2 = thymic humoral factor-γ2; QD = once per day; BIW = twice per week; TIW = three times per day; TID = three times per week.

Group, compared to 36% and 29%, respectively, in the low-dose group.81 Long-term serum ALT normalization correlated with improved hepatic function and loss of IgM anti-HDAg. Usually, recurrence of hepatitis D viremia is the rule after cessation of IFN treatment. The relapse is often delayed, sometimes occurring more than a year after the completion of the course of IFN.27 Although rare, sustained decrease in HDV replication is possible in high-dose group and is accompanied by the clearance of HDV RNA and serum HBsAg, and seroconversion to anti-HBs.81 High doses of IFN-α-2a significantly improved the long-term clinical outcome and survival of patients with CHD.92 Striking improvement in liver histology and even the disappearance of fibrosis were seen in patients who had an initial diagnosis of active cirrhosis, and a long-term biochemical response was observed.92–94 Although short-standing disease might respond better to therapy,95 predictors of response to IFN therapy are still unidentified.

Pegylated IFN

Recently, the development of pegylated-IFN (PEG-IFN) with improved pharmacokinetics has been shown to have better efficacy and convenience than conventional IFNs,96,97 providing sustained virologic response (SVR; defined as undetectable HCV RNA at least 6 months after the end of treatment) in 39% versus 17% of hepatitis C patients.97,98 Instead of fluctuating drug exposure, the sustained action of PEG-IFN reduces the viral replication that occurs on days without treatment during the
standard thrice-weekly regimen of unmodified IFN-α. In addition, the weekly dosing interval of PEG-IFN makes it a convenient therapeutic option in the treatment of patients with CHD.

There has been very little experience with PEG-IFN in the treatment of chronic hepatitis D in the past. Persistent disappearance of HDV RNA after 6 months of treatment with PEG-IFN-α-2a in a previous nonresponder to conventional IFN has recently been reported.\textsuperscript{99} In another study, 14 patients, 11 of whom had previously failed treatment with standard IFN-α, completed 12 months of PEG-IFN-α-2b (1.5 μg/kg/week).\textsuperscript{100} Six patients maintained SVR, defined as normalization of serum ALT, negative HDV RNA, and IgM anti-HDV at the end of follow-up. In a just published prospective trial of PEG-IFN-α-2b alone or in combination with ribavirin for the treatment of CHD, 38 serum HbsAg- and HDV DNA-positive patients with ALT more than 1.5 times the upper limit of normal received PEG-IFN-α-2b (1.5 μg/kg) alone as monotherapy (n = 16) or in combination with ribavirin (n = 22) for 48 weeks.\textsuperscript{91} Thereafter, all the patients were maintained on PEG-IFN for 24 weeks and followed for 24 weeks off therapy. At the end of follow-up, serum HDV RNA was negative in eight (21%) patients, and a biochemical response was detected in 10 (26%) patients. These results showed that a prolonged course of PEG-IFN-α-2b resulted in clearance of serum HDV RNA and ALT normalization in a fifth of patients with chronic hepatitis D, while ribavirin had no effect on viral clearance rate.

**Thymus-derived peptides**

Apart from IFN, other monotherapeutic approaches have been unhelpful. In a study using thymic humoral factor-γ2, none of the 11 patients achieved normal serum ALT levels, and HDV viremia relapsed in nine, one being negative at baseline.\textsuperscript{86} Although free of IFN side effects, thymosin-α1 was also not effective. Only two out of six patients achieved HDV RNA clearance and maintained ALT normalization at 12 months of follow-up after thymosin-α1 therapy.\textsuperscript{100}

**Nucleoside analogs**

Ribavirin, a nucleoside analog, is effective against RNA viruses. It has been shown to be effective in inhibiting HDV genome replication \textit{in vitro},\textsuperscript{101} but \textit{in vivo} data from a small randomized controlled trial were disappointing.\textsuperscript{102} No decrease in ALT level was observed, and all cases remained positive for serum HDV RNA and IgM anti-HD.\textsuperscript{102}

**Deoxynucleotide analogs**

Deoxynucleotide analogs have also been used to inhibit HDV replication by repression of HbsAg production. In a pilot study, 6-month famiclovir had no effect on disease activity, HbsAg levels, serum HDV RNA or lessening of the inflammation in the liver.\textsuperscript{85} Lamivudine is a potent inhibitor of HBV DNA replication, but none of the CHD patients receiving lamivudine 100 mg/day for 1 year showed any improvement in disease activity, liver histology or lower HDV RNA levels.\textsuperscript{83} Rarely, disease remission was observed in a patient after lamivudine 150 mg/day for 28 months.\textsuperscript{103} In a recent multicenter randomized controlled pilot study, only 8% of HDV patients receiving lamivudine 100 mg/day for 52 weeks achieved SVR.\textsuperscript{87} It is, thus, unlikely that lamivudine monotherapy is effective for HDV, but a recent study reported that lamivudine-resistant HBV mutants failed to efficiently support HDV secretion.\textsuperscript{104} Whether this interesting finding has any clinical implication awaits to be elucidated. Overall, available evidence does not support the use of deoxynucleotide analogs.

**Combination therapy**

Although the optimal treatment choice for CHD has not yet been clarified, certain therapeutic concepts can be derived from the experience of treating patients with chronic hepatitis C. A major advancement in treating hepatitis C has been the development of combination therapy with PEG-IFN/IFN and ribavirin.\textsuperscript{105,106} Since HCV and HDV are both hepatotropic RNA viruses, it is logical to speculate that combination therapy may also potentiate the therapeutic effect of IFN in HDV patients. However, a recent trial suggested
that ribavirin had no additive effect on viral clearance rate.91

IFN plus ribavirin
HDV, like HCV, is a RNA virus. The combination of IFN-α and ribavirin for 24 months in 19 CHD patients resulted in normalization of ALT and disappearance of HDV RNA in 42.1% and 42.1%, respectively, at the end of treatment, and in 36.8% and 21%, respectively, at the end of at least 6 months of follow-up.88 Another small randomized controlled study comparing 2-year IFN therapy with or without ribavirin in 31 CHD patients showed normalization of ALT and disappearance of HDV RNA in 57.1% and 52.4%, respectively, at the end of treatment, and in 23.8% and 23.8%, respectively, at the end of at least 6 months of follow-up in the combination group.89 None of the patients with liver cirrhosis were responsive at the end of the follow-up period.89 Unlike its overwhelming success in the treatment of HCV patients, the combined regimen is not superior to IFN-α monotherapy with regard to the clearance of HDV RNA,88,89 despite its partial effectiveness in improving the biochemical response in these patients.

IFN plus acyclovir or lamivudine
In chronic HDV patients, there is no evidence that acyclovir enhances IFN-based therapy.107 The combination of lamivudine and 16 weeks of high-dose IFN aimed at eradicating HDV was also not successful. The decrease in serum HDV RNA from baseline during treatment was not significant, and all patients with improved ALT at the end of treatment had biochemical rebound after withdrawal of therapy.84

Further clinical trials are needed to clarify whether PEG-IFN combined with other nucleoside/nucleotide analogs are more effective in treating CHD than conventional IFN-based therapy.

RNA interference
RNA interference (RNAi) has been utilized against viral infection, especially viral hepatitis. Nonetheless, among the three RNA species of HDV, i.e. genomic, antigenomic, and delta antigen mRNA, only the last can be successfully targeted by small interfering RNA (siRNA) in cell culture.108 Regarding this, it is considered that the genomic and antigenomic RNAs are resistant to siRNA because their location within the nucleus makes them inaccessible to siRNA-mediated degradation.108 In addition, the endonuclease dicer cleaves RNAs that are 100% or nearly 100% double-stranded. Therefore, despite the partial double-stranded rod-like structure of genomic and antigenomic HDV RNAs, the extended structure with only 74% base pairing confers significant resistance to dicer action.109

Prenylation inhibitors
Previous research has demonstrated that prenylation of L-HDAg, one of the post-translational modifications, is essential for viral assembly. In HDV, prenylation inhibitors could have a dual mechanism: inhibiting prenylation of a viral protein and the cellular pathways exploited by the virus.110 Animal models have shown that prenylation inhibitors are highly effective at clearing hepatitis D viremia, depending on the duration of treatment.112 Steps in acetylation, methylation and phosphorylation also reveal differential subcellular domain targeting of the HDV protein and could potentially shed light on the novel targets for the treatment of HDV. Thus, molecularly tailored drugs capable of interfering with crucial viral replicative processes would appear to have the most prospects in the treatment of HDV.

Prevention
Unlike HBV, screening of blood and blood products for HDV is considered unnecessary because HDV cannot replicate in the absence of HBsAg. Since the spread of HDV is closely linked with that of its helper virus, HBV, strategies that decrease the incidence of HBV will result in a corresponding
decrease in HDV infection, implying that the administration of universal HBV vaccination and post-exposure prophylaxis will help to eliminate HDV coinfection in the future. Education to reduce high risk behaviors among persons with chronic HBV infection will also reduce the incidence of HDV superinfection. A recent mouse model study has suggested that DNA vaccines against HDV can induce significant cellular immune responses with a T-helper 1 preference to prevent HDV superinfection.11 Since universal HBV vaccination has effectively decreased the HBV carrier rate in the general population, the development and role of HDV vaccine is therefore not so compelling.11,2

Conclusion and Perspectives

HDV can be considered as a satellite of HBV. Possibly more than any other infectious agent, the replication of HDV is dependent on redirecting host functions. Fortunately, HDV is vanishing probably owing to steps in the prevention and treatment of HBV. Improvements in socioeconomic conditions, reduced family size, changes in the behavior of intravenous drug users and in sexual practice have also contributed to the declining incidence of HDV infection worldwide. Still, HDV RNA and its replication continue to be a new and interesting issue in molecular biology. Diagnosis relies on detection of antibodies against HDAg and serum HDV RNA, as well as HBV markers. Although fraught with adverse effects, high-dose long-duration IFN is the only effective modality of CHD treatment thus far. It is hoped that in the near future, significant clarification of the processes by which HDV replicate will be made, thus offering more opportunities to treat HDV with novel approaches at the molecular level.

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