Developments In Hepatitis Delta Research

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Citation

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Abstract

The hepatitis delta virus (HDV) is the smallest human pathogen known so far. Several characteristics of the virus RNA genome resemble that of plant viroids. Since the hepatitis B virus surface antigens are part of the HDV envelope and are necessary for productive infection, HDV may be considered a satellite virus of HBV. Coinfection of the two viruses or superinfection with HDV in HBV carriers increases the risk of fulminant hepatitis and development of liver cirrhosis. At present, there is no specific treatment for hepatitis D. However, vaccination against HBV confers protection against coinfection with HDV. Although the increased rate of vaccination against HBV in developed countries reduced the prevalence of HDV, it is still a threat and remains endemic in many regions of the world. Here, we overview the epidemiology and treatment of hepatitis delta and report recent advances in the research of HDV biology.

CLINICAL AND EPIDEMIOLOGICAL FEATURES

The hepatitis delta virus (HDV) was discovered by the italian gastroenterologist Mario Rizzetto while studying liver biopsies of hepatitis B virus (HBV) infected patients (Rizzetto et al., 1977). Later, it was shown that HDV infects individuals previously infected with HBV, causing more severe hepatic lesions, and increasing the risk of fulminat hepatitis (Gorinvadarajan et al., 1984; Jacobson et al., 1985). Since the presence of HBV is necessary for the production of infectious HDV particles capable of propagating the infection, the HDV may be considered a HBV satellite virus (Rizzetto et al., 1980; Ponzetto et al, 1988).

The clinical association between these two viruses is due to the fact that the genome of HDV does not encode for its own envelope proteins. The HDV envelope consists of HBV surface proteins (HBsAg; Smedile et al., 1994), and as a consequence HDV transmission occurs only in the presence of HBV.

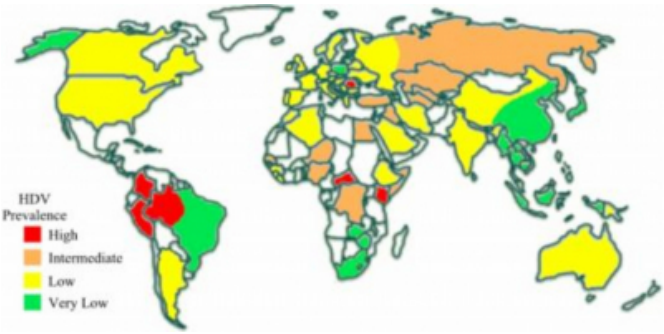
HDV replication seems to occur only in the liver, and all pathological abnormalities are limited to this organ. The lesions are similar to those observed during the course of other acute and chronic viral hepatitis. Often they consist of hepatocellular necrosis and inflamation. From the histological point of view, there are no significant differences between lesions caused by HDV and those caused by other hepatitis viruses.

The clinical course of HDV infection may be variable. In general, the observed symptoms are more severe than those associated with other hepatitis viruses. The incubation period varies between 3 to 7 weeks. Following this period, the first symptoms can be detected, including fatigue, lethargy, and nausea. It is estimated that 60% to 70% of all hepatitis delta chronic patients will develop chirrosis. This percentage is about three times higher when compared to the percentage that is observed in hepatitis B patients (Rizzetto et al., 1983). The frequency of HDV associated fulminant hepatitis, the more severe form of the acute disease, is 10 times higher than the observed for other viral hepatitis. Fulminant hepatitis is usually associated with hepatic encephalopaties that, in the most severe cases are characterized by somnolence, abnormal behavior, and coma. The mortality associated with fulminant hepatitis is about 80%, independent of the treatment (Purcell and Gerin, 1990).

Seroprevalence studies in individuals positive for HBsAg, show a non-uniform worldwide distribution of HDV (Ponzetto et al., 1985; see fig. 1).

Figure 1

Figure 1: HDV prevalence in the world.



Serotype I is more frequently found in the USA, Europe, North of Africa, Asia and Southern Pacific. Additionally, serotype II is frequently reported in Asia, namely Japan and Taiwan, and serotype III is mainly confined to South America. At present, about 5%-10% of the estimated 400 million individuals infected with HBV are thought to be HDV carriers. The regions with higher prevalence are the Mediterranean basin, including the Middle East, some Central African countries, the Amazonas basin, and some Asian countries including Japan and Taiwan. In these two countries some endemic regions, with a prevalence of more than 30% in chronic hepatitis patients, have been detected (Huo et al., 1997; Nakasone et al., 1998). In contrast, in Italy and other developed countries the disease seems to be declining due to the high overall rate of vaccination against HBV (Wu et al., 1995). In Portugal, according to the few studies published so far, HDV prevalence may reach about 17% of all chronic HBsAg carriers (Ramalho et al., 1987). Moreover, about 8% of all viral hepatitis seem to be associated to HDV (Velosa et al., 1993). Delta hepatitis affects individuals of both genders and all age groups. The most common route of infection seems to be the direct contact with blood or blood products from infected carriers. In this context, dissemination between drug addicted groups represents a dangerous public health problem (Hansson et al., 1982; Oliveira et al., 1999). Although HDV and HBV share similar routes of transmission, some epidemiological studies carried out in the Amazonas suggest a possible role of insect transmission in the dissemination of HDV (Fonseca et al., 1994; Arboleda et al., 1995).

There is no specific treatment for delta hepatitis. As observed with other forms of acute and chronic virus hepatitis, immunosupressive therapies are of very limited if any clinical value, (Rizzetto et al., 1983). The use of interferon may inhibit virus replication (Hoofnagle et al., 1987; Rizzetto et al., 1986; Thomas et al., 1987) but, as it also happens with HBV, the potential benefits of such a treatment are transient. More recently, it has been reported the cure of a chronic hepatitis delta patient after 12 years of daily treatment with alfa interferon (Lau et al., 1999a). Unfortunetely, treatment with alfa interferon may be associated, in some patients, with the appearance of more severe symptoms (Crosignani et al., 1999). A number of antivirus drugs have been tested in vitro and in chronic HDV carriers. Among them, lamivudine (3–thiacytidine), a nucleotide analogue that seems to be capable of inhibiting HDV replication, was thought to constitute a promising alternative (Lau et al., 1999b). However, HDV lamivudine resistant mutants have already been reported as a consequence of treatment with this drug (Rizzetto, 1999). A combined therapy of lamivudine with other antivirus drugs, namely ribavirine, may prove to be more succesful. However, today there is no valid therapy for delta hepatitis.

THE BIOLOGY OF HDV

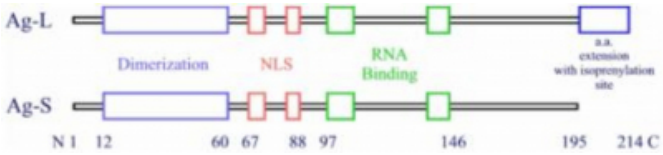
Research on HDV biology has long been made difficult due to the lack of a cellular system capable of replicating the virus. However, since 1993 the construction and subsquent availability of stable hepatitis delta-expressing hepatoma cell lines (Cheng et al., 1993), greatly improved our knowledge on the cellular and molecular biology of this small pathogen. The HDV virion has a spherical structure of about 36 nm diameter. The virus envelope consists of cellular lipids and hepatitis B virus surface antigens (HBsAg). The virion core ribonucleoproteins are encoded by the virus genome. The HDV genome consists of a circular single-stranded RNA molecule of about 1,700 bp that displays a high degree of internal base-pairing (aprox. 70% of the genome). The HDV RNA secondary structure is thus comparable to that of plant viroid RNAs (Taylor, 2003; Lai, 1995). One single ORF was found on the HDV genome. Indirect evidence, mainly obtained with the use of inhibitors such as -amanitin, suggests that transcription of this ORF is driven by cellular RNA polymerase II and, as a result, a 800 nucleotide mRNA molecule is synthesized (Modahl et al, 2000; Moraleda and Taylor, 2001). Translation of this mRNA results on the production of the small form of the delta antigen (S-HDAg), a 195 aminoacids protein of about 24 KD that is localized in the nucleus of infected cells. During HDV replication an RNA editing mechanism occurs in the antigenome molecule, converting an amber stop codon UAG to a UGG tryptophan codon. As a result, the ORF is extended by 19 additional aminoacids and a 214 aminoacid protein of about 27 KD, the large form of the delta antigen (L-HDAg; Taylor, 2003; Lai, 1995), is synthesized. This large form is also localized in the

nucleus of infected cells. In conclusion, both antigens display the same cellular localization and share the same aminoacid sequence, with the exception of the 19 additional aminoacids present in the carboxyl terminus of the large form.

Several specific structural domains were found in the delta antigens, including a nuclear localization signal (NLS) and leucine zipper RNA binding domains (Figure 2).

Figure 2

Figure 2: Localization of structural domains in HDV antigens.



The NLS is localized between aminoacids 67 and 88 and consists of a bipartite sequence rich in basic residues (Xia et al., 1992). The RNA binding domain consists of two arginine rich sequences (97-107 and 136-146). Both segments were found to be necessary for in vitro binding to HDV RNA (Lee et al., 1993). It has been shown that the HDAg RNA binding properties are specific for HDV RNA, and are necessary for activation of virus replication (Lee et al., 1993). The last four of the 19 additional aminoacids present in the carboxyl terminus of L-HDAg are thought to constitute an isoprenylation signal. These last 19 aminoacids are necessary for interaction with HBsAg. Experiments performed by Lee et al. (1994, 1995), where these last 19 aminoacids in the carboxyl terminus of L-HDAg where added to c-Hras rendering it co-secretable with HBsAg. However, c-Hras did not become co-secretable when only the 4 aminoacids containing the isoprenylation signal were added.

In spite of all the similarities in the sequence and structure between both delta antigens, they display distinct biological roles in relation to HDV replication. S-HDAg is essential for virus replication (Kuo et al., 1989) whereas L-HDAg seems to inhibit replication by a trans-acting supressor mechanism (Chao et al., 1990). In addition, L-HDAg plays an essential role in virion packaging (Chang et al., 1991; Ryu et al., 1992). Relating structure to function in HDAgs we can briefly say that the second RNA binding domain of S-HDAg is necessary for initiation of replication (Lazinski and Taylor, 1993) whereas the first RNA binding domain of L-HDAg is responsible for inhibition of replication (Lee et al., 1995). Since S-HDAg is packed into virions only in the presence of L-HDAg, this also may suggest that direct interaction with HBsAg is mediated by the large form of the delta antigen (Chen et al., 1992).

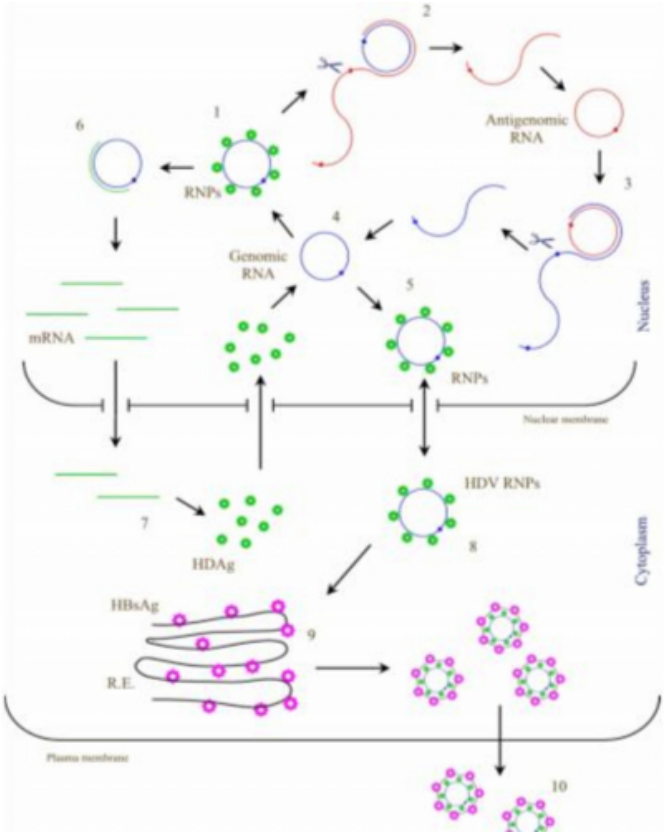
Both virus RNA and proteins are predominantely localized in the nucleus of host cells suggesting that HDV replication occurs in this cellular compartment (Cunha et al., 1998; Gowans et al., 1988).

It is not still clear whether, after infection, HDV RNA is transported to the nucleus alone or together with HDAgs. Since the structure of HDV RNA is very similar to that of plant viroids, and the laters enter the nucleus devoid of any protein (Harders et al., 1989), it is possible that HDV RNA possesses an intrinsic capacity of nuclear import. However, more recent data seem to support a model where the whole virus RNP, but not the naked RNA, is transported to the nucleus upon infection (Chou etal., 1998). Thus, import of HDV RNA to the nucleus is likely to be the first biological function of delta antigens in the virus life cycle.

HDV replication is totally independent of any HBV sequence or function. Upon entry into the cell, HDV RNPs are transported to the nucleus and the RNA is replicated by one or more cellular RNA polymerases (Mcnaughton et al., 1991; Filipovska and Konarska, 2000; Modahl et al., 2000). There are several indirect lines of evidence suggesting the involvement of at least RNA polymerase II. According to the widely accepted model for HDV replication (Figure 3), the RNA genome serves as template for the synthesis of new molecules by a rolling circle mechanism. RNA replication results in the production of multimeric antigenomic molecules. These nascent antigenomes harbor a 100 nucleotide ribozyme domain, which self-cleaves the RNA multimers at precise monomeric intervals. Two self-cleavage events give rise to monomeric linear antigenomes that self ligate thus producing a circular molecule called the antigenome (Taylor, 2003).

Figure 3

Figure 3: Schematic representation of the HDV replication cycle. 1- The HDV genomic RNA serves as template for synthesis of both multimeric antigenomic molecules and mRNA (6); 2- RNA antigenomic multimers are self-cleaved at monomeric intervals and circularize; 3- the antigenomes serve as template for synthesis of multimeric genomic RNA molecules; 4- RNA genomic multimers are self-cleaved at monomeric intervals and circularize; 5- HDV RNPs assemble and are exported to the cytoplasm where they meet HBsAgs (8 and 9); 10- mature HDV virions are produced and secreted; 7- translation of mRNA results in the synthesis of delta antigens which are imported to the nucleus. See text for details.



Recent studies indicate that S-HDAg can act as a RNA chaperone in the cleavage and ligation reactions mediated by HDV ribozymes (Huang and Wu, 1998). RNA editing is thought to occur on the antigenome by means of a cellular adenosine deaminase, most likely the small form of ADAR 1 (Jayan and Casey, 2002a; Wong and Lazinsky, 2002), which is responsible for the conversion of the adenosine into an inosine in the UAG stop codon (Casey and Gerin, 1995). Editing can additionally occur at other nucleotides on the antigenome, being responsible for the mutant RNA species that can be found in infected cells (Jayan and Casey, 2002b). Following genome replication it is likely that the resulting monomeric antigenomes serve as templates for the subsequent synthesis of new RNA genomes by a similar rolling circle mechanism (Taylor, 2003).

The HDV RNA, delta antigens and virus RNPs are almost exclusively localized in the cell nucleus (Cunha et al., 1998). In fact, inside the cells the HDV displays a diffuse nucleoplasmic distribution with accumulation in foci and nucleolar exclusion. These foci contain both virus RNA and antigens, but apparently do not correspond to preferential sites where RNA synthesis and processing occurs (Cunha et al., 1998). In contrast, the HBsAgs, which are necessary for HDV virion packaging, are glycoproteins associated to the endoplasmic reticulum and thus are located exclusively in the cytoplasm. How do these two components of HDV, which are spatially separated in infected cells, meet to assemble infectious virus particles? An explanation for this intriguing observation was given in a work by Tavanez et al. (2002). The authors showed that HDV RNPs shuttle between the nucleus and the cytoplasm, and that the signal for export is present on virus RNA. In the presence of HBV, HDV RNPs interact with HBsAgs to form infectious virus particles. If the HBsAgs are absent then HDV RNPs are rapidly reimported to the nucleus due to the bipartite nuclear localization sequence present in HDAgs.

As pointed before, the HDV RNA bears an autocatalytic ribozyme activity. This feature of the virus genome may prove to be an important tool in the search for a specific anti-HDV therapeutic strategy. In recent years, ribozymes revealed a great therapeutic potential due to its capacity to cleave RNAs that are harmful to normal cell functions. These properties may be useful in gene therapy for some cancer and virus diseases, and are the focus of intense investigation. As an example, the use of ribozymes for treatment of hepatitis B has been reported (Welch et al., 1997). These authors observed a 83% decrease in the number of virus particles in the liver of infected patients. In spite of the lack of precise knowledge of the secondary and tertiary structure of the HDV ribozyme, it has been extensively studied and modified in vitro in order to make it act in trans and thus make it potentially useful for gene therapy (Been, 1994; Kawakami et al., 1996). In conclusion, a deeper knowledge of HDV biology, namely the properties of its RNA, may contribute to the development of novel and promissing approaches to the treatment of the chronic disease.

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