A Modern View of The Pathogenesis and Treatment of HDV Infection

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A Modern View of The Pathogenesis and Treatment of HDV Infection

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ABSTRACT

Hepatitis Delta is severe and rapidly progressing form of viral hepatitis which leading to the liver cirrhosis and this infection is still an unresolved global health problem. The treatment of delta-virus infection is an unresolved problem. The application of the interferon preparations in case of delta-infection does not guarantee the efficacy of the treatment in the majority of patients, does not provide the stability of the elimination of the virus and gives a set of side-effects. New preparations with other mechanisms of the actions are now developing. These are nucleosides analogues and preparations with the specific mechanisms of action like Myrcludex B, Lonafarnib, REP 2139 which are in process of clinical tests. The combined therapy by the interferons and the nucleoside analogues seems to be perspective.

Viral hepatitis remains the most urgent issue for the global public health care. The infectious diseases become cause of death for above 4 million people every year, while about 1 million people die from hepatitis B (HBV) and C (HCV) every year in the world, and even more infected people lose their ability to work. Losses connected with the viral hepatitis compose a significant share of the economic damage caused by the most common infectious diseases. Currently the role of understudied and poorly treatable mixed infection of hepatitis B with the delta agent is growing against the background of the rapid progress in treatment of the viral hepatitis C.

In the world there are 400 million people infected with the hepatitis B virus, of which 15-25\% (750 thousand) is fatal every year due to the liver cirrhosis complications and hepatocellular carcinoma. The share of HBV patients with a concomitant delta agent that dramatically complicates its natural course varies from below 1\% to above 10\% in different populations. Worldwide, 20 million people can be infected with the hepatitis Delta virus [1,13].

In 1977, the Italian Doctor M. Rizetto reported discovery of a new Delta antigen in the patients with chronic hepatitis B [42]. Later it was defined that this antigen belongs to another pathogen of hepatitis, namely HDV, while the HDV virus genome was cloned and sequenced in 1986 [50].

\textbf{Epidemiology.}

The anti-HDV antibodies detection rate serves as an indicator of the chronic HDV prevalence. The regions can be conditionally assigned to one of four zones in terms of the Delta infection prevalence in the patients with hepatitis B: zones of high endemicity - the anti-HDV antibodies frequency composes above 60\%; zones of medium endemicity - the
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Virus Properties. HDV is a spherical formation with a diameter of 28-39 nm, in average of 36 nm. RNA - the genome and the HDV antigen - is located at the center of the particle. It is covered with HBsAg from above. The HDV genome is a single-stranded, circular RNA consisting of an average of 1,700 nucleotides [33]. The HDV particle contains a delta antigen that exists in two forms: a large Delta antigen (L-HDAg) consisting of 214 amino acids and having a mass of 27 kDa and a small Delta antigen (S-HDAg) with a length of 195 amino acid residues and a mass of 24 kDa [54]. Both N-terminus forms have a helix, necessary for dimerization. Delta antigen dimers have an
arginine-rich motif that allows it to bind to viral RNAs. However, the L-HDAg extended C-terminus have four unique residues of cysteine serving as targets for farnesylation. After this post-translational modification, L-HDAg can interact with HBV surface proteins (HBsAg) and thereby facilitating the assembly of new virus particles. Small Delta antigen is necessary for viral RNA replication and functions in the early stages of infection [14].

The total amount of RNA HDV in one hepatocyte can reach from 100,000 to 300,000 copies, which indicates a high rate of replication [8].

The HDV pathogenesis has the unique ability to use human RNA-dependent RNA polymerases to transcribe its own RNA without formation of the intermediate forms of DNA.

**Cell penetration and viral replication cycle:**

HDV hepatotropism and its ability to reproduce within hepatocytes is associated with co-infection HBV. The initial stages of the HDV interaction process with the hepatocyte take place due to HBsAg.

The functional receptor for HBV and HDV is counter-transport peptide of the sodiumtaurocholate (NTCP). NTCP is located in the basolateral membrane of hepatocytes and its main role in vivo is transportation of the bile salts from portal blood to hepatocytes. This process is known to be vital for maintaining biliary acid homeostasis. NTCP is responsible for the majority of the hepatic bile salts, as well as for transportation of certain drugs and xenobiotics [20,34].

Viral infection is supported by amino acids involved in binding of bile acids. The interaction between NTCP and HBV/HDV is mediated by 75 N-terminal amino acid residues in the PreS1 domain of viral surface proteins and the binding sector on NTCP located on the outer layer of the cell membrane. Since the HBV and HDV shell structure is very similar, it can be assumed that the mechanisms of attachment to the target cell and penetration into it will be common for these viruses. In fact, most of the currently available information regarding the mechanisms of HBV penetration into the cell is obtained from HDV infection models [3,21].

During replication, antigenomic RNA is found only in the nucleus and is synthesized in the nucleolus, while genomic RNA molecules synthesized in the nucleoplasm can be involved into another replication cycle in the nucleus or exported to the cytoplasm to assemble new viral particles [38].

Three enzymatic activities are necessary for HDV RNA replicating:

- polymerase activity for the oligomeric chains synthesis on ring matrices, when antigenomic matrix is synthesized in the genomic matrix in the first stage and the antigenome acts as a matrix for synthesis of the genome in the second stage;
- ribozyme-dependent RNA-type activity for cutting these chains into monomers;
- ligase activity for closing the monomers in ring [12].

Unlike some RNA viruses with larger genomes, HDV has no its own RNA-dependent RNA polymerase. Moreover, unlike other satellite viruses, HDV does not use the helper virus polymerase (that is, a virus, only in the presence of which formation of HDV virions is possible) and therefore relies entirely on the enzymes of the host cell. There is some evidence that host cell RNA polymerase II is involved in HDV replication. Obviously, HDV is able to make DNA-dependent RNA polymerase work with RNA. The mechanisms for such a switch are unclear to a significant extent. S-HDAg apparently is involved in switching the RNA polymerase II specificity; it can bind to RNA polymerase II and enhance transcription either directly stimulating elongation or leveling inhibitory
effects. DNA-dependent enzyme probably works with genomic RNA due to its partially double-stranded rod-like structure [3,38].

The antigenomic RNA is prepared during replication under influence of the cellular enzymes - Adenosine deaminase impacting RNA (ADAR1), which catalyzes the ST-stop codon mutation on the tryptophan codon (UGG) S-HDAg, which results in approximately 30 amino acid elongation (depending upon genotype) [39]. The resulting mRNA leads to formation of the L-HDAg protein containing S-HDAg with a C-terminal amino acid elongation. This extension contains a signal on nuclear export and HBsAg binding fragment by the sector prenylation in Cys211, which is post-translationally farnesylated by the cellular farnesyl transferase. Farnesylated L-HDAg is required for HDV assembly, as it interacts with the cytosolic HBsAg loop during shell formation [19,24].

Thus, membrane formation around HDV ribonucleoprotein requires farnesylation of the L-HDAg C-terminal sector, since it controls the interaction with the HBsAg S-sector. However, farnesylation of the C-terminal sector requires L-HDAg prinylation. HDV assembly is impossible in absence of these mechanisms.

HDV virion formation requires HDV ribonucleoprotein coating with S- and L-HBsAg, therefore HDV particles assembly is only possible in HBV co-infected cells. There are many questions having no answers concerning the HDV particles assembly and the cell exit. HDV particle assembly is possible only in co-infected HBV cells, since HBsAg is required for wrapping. However, HBsAg can also be synthesized by the defective HBV genomic integrals which are not encoding complete pregenomic RNA HBV, but still encoding HBV shell proteins with self-assembly [16]. Functional viral particles are wrapped by both L-HBsAg and S-HBsAg. Fundamentally, HDV coverage requires only S-HBsAg (unlike HBV, which also requires L-HBsAg), since L-HBsAg is crucial for the HDV virus infectivity; the particles covered only by S-HBsAg remain non-infectious, although may contribute to "viral load" in the infected patients. Further on the wrapped particles are secreted from the cells. It remains unclear whether this happens by the classical secretion or through the multivesicular bodies (for instance, for HBV) [48].

Unlike HBV requiring the cytoplasmic HBsAg domain for the particles release including the connecting sector between PreS1 and PreS2, HDV does not need this. Based on this, it was assumed that HDV predominantly releases sub-virus particles through the Golgi apparatus, rather than through a multi-vesicular body, like HBV [3].

**Scheme No.1. HDV Replication in the Hepatocyte.**

1. HDV virus in Disse space; 2. NTCP interaction with PreS1 denome HBsAg; 3. HDV genome penetration into the hepatocyte cytoplasm; 4. HDV genome penetration into the hepatocyte nucleus; 5. HDV genome replication; 6. synthesis of HDV mRNA by antigenomic RNA HDV; 7. Pre S 1 and Pre S 2 domains synthesis using HBV ccc DNA; 8. HDV genome released into cytoplasm; 9. S-HD Ag and L-HD Ag synthesis using HDV mRNA; 10. L-HDAg prinylation; 11. HDV genome wrapping with S-HD Ag antigens and prenylated L-HD Ag; 12. HDV genome wrapping with S-HD Ag antigens and prenylated L-HD Ag in HBsAg; 13. ready HDV virion released from the hepatocyte.
As noted, taurocholate sodium peptide (NTCP) is responsible for transportation of the bile salts; however, in presence of HBV and HDV viruses, it can also be used to penetrate the virus genetic material into the cell. Nevertheless, it remains unclear whether these two NTCP functions are independent or interfere with each other.

In his research, Huan Yan discovered that the pre-S1 domain actively blocks absorption of NTCP bile salts substrates. This, in turn, leads to accumulation of the bile salts substrates, which inhibited the HBV and HDV penetration into the cell to varying degrees. These results in deeper understanding of interaction between the shell proteins of HBV and NTCP proving that bile acids and derivatives of such may contribute to blocking of HBV and HDV.

The studies of the authors proved the tauro-conjugate form of ursodeoxycholic acid (TUDCA) to be an inhibitor of HBV and HDV viral infections. Given the above mechanism, several clinical observations proved that long-term use of the bile salts, in particular, TUDCA, leads to improvement in the liver function and affects the viral load in patients with hepatitis B.

In his studies, Zhang Z, described the interferon (IFN) promotion during HDV infection and assessed the IFN effect on the HDV replication. The author’s study analysis proved that active replication of HDV, but not of HBV, induces IFN-β and IFN-λ in the infected hepatocyte cells; however, this impacts insignificantly the HDV replication. During the prolonged infection, IFN levels gradually decreased in both HepG2NTCP cell lines and HepaRGNTCP cell lines of the study models. According to the author, this mechanism explains adaptation to interferons in the HDV replication. In addition, IFN Type I or III treatment does not block HDV replication.

However, it should be noted that in his study, Katashiba Y., indicates that IFN is mainly stimulated in DNA containing infections, while IL-12 induction predominates in RNA containing infections. Since HDV consists of a single-stranded RNA molecule, it is not expected that it will stimulate IFN.

We believe that the above disagreements of the authors can be explained by the fact that replication possibly induces IFN synthesis inside the hepatocyte in case of the HBV virus, while HBV suppression blocks IFN synthesis. Further, HDV is not able to induce IFN, namely induces IL-12, which in turn indirectly induces IFN-gamma; however, by this time HDV becomes resistant to IFN. In addition, HDV blocks IFN signaling to a nearby hepatocyte.

Clinical Findings

Studies of the recent 40 years have proved that a significant part of liver diseases, previously considered as the infection result with the hepatitis B virus (HBV), are actually the result of simultaneous infection with B and Delta viruses (HDV). The disease course, as a rule, is characterized by a particular severity. However, a Delta infection exists in several forms that differ in clinical characteristics, frequency, outcome. The virus can cause conditions that are similar to the acute viral hepatitis (AVH), as well as to the typical forms of chronic hepatitis (CH) and liver cirrhosis (LC). The asymptomatic carriage of HDV is also possible. WHO estimated that at least 20 million of HBsAg carriers in the world simultaneously had Delta virus. Currently, this number is estimated at 25 million.

According to B. Pinarbasi Simsek et al., the HBsAg and HDVRNA concentrations in the patients with HBV and HDV infection is reliable and correlated positively (p=0.043). All patients examined had various HDV viral load not correlating with biochemical and histological activity or the stage of fibrosis. Serum transaminase
concentration and histological had no correlation between HBVDNA-negative and positive patients with chronic HDV (p > 0.05), while HBsAg concentrations had minor difference in patients with positive and negative HBVDNA (p > 0.05). HBsAg levels were significantly higher in patients with HBeAg-positive chronic HDV than in patients with HBeAg-negative chronic HDV, but being the same levels in patients with chronic HDV and HBeAg-negative chronic HBV. The histological activity and fibrosis stage were significantly higher in chronic HDV than in patients with chronic HBV; however, such were not affected by the HDV and HBV viremia levels [4].

Background in the Republic of Uzbekistan. Musabaeva E.I. et al. presented the effect of IL28B-gene genetic variations on the risk of the liver cirrhosis developing and possibility to predict the disease outcome in HDV-infected patients. The genotype rs8099917 in IL28B locus TaqMan SNP was defined by genotyping in 94 individuals, including 72 patients with liver cirrhosis (LC) associated with HDV infection aiming to assess the effect of IL28B rs8099917 SNP among the studied groups. A group of 22 patients with HBV LC etiology was taken for comparison. The study results proved that the genotypes distribution in HDV-induced LC was significantly different from the HBV-etiolog LC, both as per rs8099917 TT genotype (p<0.001) and as per rs8099917 TG genotype (p<0.001). Traditional risk factors for LC (sex, age, viral load, comorbidities) had insignificant effect on the chronic viral hepatitis outcome. The studies results proved that the genotypes distribution in HDV-induced LC was significantly different from the HBV etiology LC, both as per TT-genotype and TG-genotype. Thus, genetic analysis is a promising method for non-invasive diagnosis of the risk of LC developing [31].

Interesting studies were conducted for the viral hepatitis B and mixed hepatitis in children. Inoyatovoy F.I. et al. conducted the clinical analysis in children with the chronic HBV, having refractory variant of anemia of inflammation (AI), the pathogenetic manifestation of which was development of iron overload syndrome (IOS). It was revealed that, the disease progressive forms frequency increased with persistent prevalence of asteno-vegetative, hemorrhagic syndromes and severe hepatosplenomegaly secondary to increasing severity of IOS. At the same time, the leading biochemical syndromes were: presence of the cytolsis with prolonged hyperfermentemia, endotoxemia and mesenchymal-inflammatory syndrome [28].

The frequency of clinical manifestations of delayed sexual development (DSD) and the functional status of the pituitary-gonadal system in boys with chronic hepatitis B (chronic HBV) was studied depending on the activity and duration of the disease. The incidence of DSD among the examined boys composed 60.4%. The serum basal levels of gonadotropic and sex hormones - FSH, LH, T, E. were studied. It was proven that chronic HBV as a chronic infectious process negatively affects development of the reproductive health of boys [29].

74.6% of cases (versus 22.2% of healthy children) of examination of patients with chronic hepatitis B revealed psychological changes that were characterized by impairments in the intellectual (66.9%), emotional (66.1%) and behavioral (71.6 %) spheres. At the same time, the level and direction of psychological manifestations were conditioned by the age features of the sick children development [26].

The activity of lipid peroxidation (POL) and the antioxidant system (AOS) effectiveness in patients with chronic mixed hepatitis (chronic MH) with persistence of HB-, HC- and HD viruses were studied. It was revealed that mixed infections with hepatotropic viruses exacerbate membrane-destructive processes in cells, contributing to development of severe forms of the disease. Prolonged hyperfermentemia and disruption
of the relations between the POL/AOS processes indicators as well as development of the persistent intensification of the POL reactions, especially with triple (B + C + D) infection are characteristic biochemical signs of chronic mixed hepatitis (CMH) in children. As a result, destabilization of the membrane lipid structure was manifested by increase in cholesterol, triglycerides and decrease in phospholipid levels (27).

The next goal of the authors' work was to study the clinical and biochemical features and marker profile in children with the chronic viral mixed hepatitis (chronic VMH). 480 patients aged from 3 to 14 years were observed. Among them, 120 (25.6%) children had mixed hepatitis, 86 (71.7%) had HBV and HCV markers, 34 (28.3%) had HBV, HCV and HDV markers. Virological verification was performed by ELISA and PCR. The results of the clinical examination of children, with chronic viral mixed hepatitis allowed to conclude that the disease severity depends upon the persistence and replication of two/three viruses. This is clearly reflected in the high frequency and severity of clinical syndromes, such as asthenovegetative, hemorrhagic and cholestatic with prevalence of large sizes of the liver and spleen in children, infected with two and especially three viruses. The clinical symptoms are 1.9 times less frequent than those in patients with mixed infection and are relatively moderate if there is only one virus (HBV). This is probably conditioned by absence of the additional damage factor (other viruses) and ability to integrate HBV into the hepatocyte genome. Cytolysis, endotoxemia and cholestasis syndromes are the leading biochemical indicators of liver damage in chronic viral mixed hepatitis [25].

In 2003-2004, the studies of T.A. Daminov et al. defined HBV genotypes in children suffering from chronic viral hepatitis B (HBV). All DNA-HBV-positive patients (n=40) were genotyped for the hepatitis B virus. Studies proved that children suffering from chronic viral hepatitis B were infected with various HBV genotypes, with prevalence of the O (77.5%) and A (15%) genotypes [10].

The studies of Khikmatullaeva A.S. (32), presents incidence of the latent HBV infection. The selective screening studies were conducted in various regions of the Republic aiming to diagnose and assess the viral hepatitis prevalence rate. In total, 1,645 blood samples of conditionally healthy individuals never consulted for liver disease before were examined for presence of HBsAg and AntiHCV in the blood plasma. According to the author, the highest percentage of HBs antigenemia detection was in the Southern, Southwestern and Central regions, where positive results composed on average 12%. Whereas, positive HBsAg results averaged 10% in the Eastern, Northern and Northwestern regions. However, these patients were never examined for presence of AntiHDV unfortunately.

Possible Therapy. Timely therapy for HDV is very important, since the complex of therapeutic measures aiming to reduce the fibrotic processes progression in the liver contributes to prolong the patients' life and preserve the ability to work. According to many researchers, currently interferon is the only approved drug in treatment of HDV. However, there are few data on the effect of interferons on HDV in human hepatocytes.

The studies of Giersch K. evaluated the effect of pegylated interferon alpha (peg-IFNα) and lambda (peg-IFNλ) compared with the HBV polymerase inhibitor entecavir (ETV) on all markers of HDV infection. After infection of mice with HDV virus, HDV RNA was detected by PCR intrahepatic. Peg-IFNα and peg-IFNλ were found to reduce HDV viremia (1.4 log and 1.2 log, respectively) and serum HBsAg levels (0.9-log and 0.4-log, respectively). Both interferons significantly reduced intrahepatic levels of genomic and antigenic HDV RNA. Entecavir-mediated suppression of HBV
replication (2,1-log) had no significant effect on the HBsAg levels and HDV performance or release of such from the hepatocyte. Reduction in viremia reflected the intrahepatic decline in all HDV markers, including the antigenomic pattern, indicating that intracellular HDV clearance was achieved [18].

New directions in treatment of the chronic HDV is development of drugs that inhibit binding of the Delta agent and the hepatitis B virus, for instance, Myrcludex B. The mechanism of the substance action is ability to firmly bind to a specific hepatocyte receptor, NTCP, located on the cell surface, which in turn prevents the contact of HBsAg with the hepatocyte, which preventing the viral genome from penetrating into the cell. Myrcludex B is hepatotropic in nature and specifically binds to NTCP on the cell membrane and is accumulated on the sinusoidal membrane of hepatocytes after intravenous or subcutaneous administration. Myrcludex B inhibits NTCP bile acid transporter function, indicating HBV/HDV receptor blockade [36].

The Myrcludex-B scheme of action.

![Scheme No.2. The mechanism of the Lonafarnib action.](image-url)

Taking into account the mechanism of the Myrcludex B action, it can be assumed that the medicine is able to block transportation of the bile acids, while its side effects may be associated with the impaired bilirubin metabolism.

In addition, a drug, Lonafarnib, is currently studied. It affects the post-translational modification processes of the Delta agent antigens, in particular, C-terminus of the L-HDVAg molecule, which links the HDV RNA nucleocapsid to the HBsAg virus. Lonafarnib prevents farnesylation of the C-terminal residue of Cys211 in L-HDAg, which are directly related to the HDV assembly. Consequently, unfarnate L-HDAg is not able to bind to HBsAg. HDV gene not wrapped by HBsAg cannot escape from the hepatocyte and leads to decrease in hepatocyte infection in the liver [43].

However, the Lonafarnib effect indicates that the inhibiting farnesylation process can accelerate cytotoxic effects in the hepatocyte. Replicated HDV genomes, intermediate products within the hepatocyte or enhanced immune-mediated death of hepatocytes are accumulated.

Scheme No.2. The mechanism of the Lonafarnib action.
Analogs of nucleotides and nucleosides, as mentioned above, are ineffective in suppressing the replication of HDV RNA. However, this therapy should be considered for the patients with active HBV DNA replication.

The newest drug REP 2139, the mechanism of action of which is aimed at blocking of the ready HBV or HDV virion release from the infected hepatocyte [43].

The REP 2139 scheme of action:

1. HDV virus in Disse space;
2. NTCP interaction with HBsAg PreS1 domain;
3. HDV genome penetration into hepatocyte cytoplasm;
4. HDV genome penetration into hepatocyte nucleus;
5. HDV genome replication;
6. HDV mRNA synthesis by antigenomic RNA HDV; 7. Pre S1 and Pre S2 domains synthesis using HBV cccDNA; 8. HDV genome released into cytoplasm; 9. S-HD Ag and L-HD Ag synthesis using HDV mRNA; 10. L-HDAg prenylating; 11. HDV genome wrapping with S-HD Ag antigens and prenylated L-HD Ag in HBsAg; 12. HDV genome wrapping with S-HD Ag antigens and prenylated L-HD Ag in HBsAg; 13. No release for HBsAg thus for HDV virion. HDV Genome accumulation in Cytoplasm.

The effect of REP 2139, like the effect of Lonafarnib, can lead to accumulation of the replicable HDV genomes, intermediate products within the hepatocyte, or enhanced immune-mediated death of hepatocytes.

Discussion

There are no results on HDV genotypes in the Republic of Uzbekistan. Once the level of the HDV infection endemicity is established and the HDV genotypes circulating...
in the republic are defined, it will be possible to predict the clinical course and outcomes of the disease with mixed HBV + HDV infection.

It is known that IFN-α and INF-γ induce when DNA containing viruses are infected, while IL-12 induction prevails when RNA is infected with viruses. Induction of IFN-α and INF-γ is also detected with the HDV infection, although endogenous interferons do not affect the replication of HDV. The question of the mechanism and pathogenic significance of the induction of IFN-α and INF-γ in the HDV infection remains open.

The latest medicines developed to date do not completely resolve the problem in treatment of the patients with HBV + HDV and elimination of the HDV virus from the body. These medicines have only a temporary effect, but exhibit side effects. Myrcludex B is able to block transportation of the bile acids, while its side effects may be associated with the impaired bilirubin metabolism. At the same time administration of the Lonafarnib and REP 2139 will cause accumulation of the replicated HDV genomes, intermediate products inside the hepatocyte, or enhanced immuno-mediated death of the hepatocytes.

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