Review

Virology and clinical sequelae of drug-resistant HBV in HIV–HBV-coinfected patients on highly active antiretroviral therapy

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Several of the nucleoside/nucleotide analogues used to treat HIV also inhibit HBV replication, with lamivudine being the oldest of this group. Thus, prior to licensing of tenofovir, many HIV–HBV-coinfected individuals received lamivudine as the only drug active against HBV as part of an anti-HIV regimen, which set the stage for the emergence of drug-resistant HBV. In coinfection, lamivudine-resistant HBV develops more rapidly than in HBV-monoinfected persons, but it is not known if this is true for the newer agents. Owing to overlapping reading frames of the HBV polymerase and surface antigens, drug-resistant changes in HBV Pol can lead to mutations in the envelope. This review will discuss studies of drug-resistant HBV in HIV-infected persons including drug-resistant mutations that have been identified and clinical sequelae of these mutations.

Introduction

For the estimated 4 million HIV-infected individuals who are coinfected with HBV, drug-resistant HBV is a common problem because several of the nucleoside/nucleotide analogues used to treat HIV are also active against HBV. This dual activity of drugs is especially problematic when the HBV status is not known prior to administering HIV therapy because anti-HBV drugs with a low genetic barrier to resistance, such as lamivudine, might be given as part of an anti-HIV regimen. Once drug-resistant HBV emerges, it can be difficult to treat because only a few classes of anti-HBV drugs are available and cross-resistance between the drugs, and even between classes, occurs.

In HIV-infected individuals, the treatment of HBV is important because HIV increases the risk for liver-related mortality [1]. Furthermore, liver disease is a leading cause of mortality in HIV-infected patients on highly active antiretroviral therapy (HAART). In the D:A:D study, which followed 23,441 individuals with HIV who received HAART for a median of 3.5 years, there were 1,246 (5.3%) deaths [2]. The second leading cause of death was liver disease, which accounted for 14.5% of all deaths. About 25% of the liver deaths could be attributed to an HBV infection. For this reason, hepatitis B should be treated and consideration should be given to minimizing the risk of drug-resistant HBV in HIV-infected individuals. In this article, we will review what is known about development of drug-resistant HBV in HIV-infected individuals on HAART and discuss the clinical implications.

Virology

Although HBV is a DNA virus, it has an RNA intermediate and an error-prone reverse transcriptase; thus, substitutions are made at each base daily allowing for the development of drug-resistant variants (see article by Locarnini and Yuen in this issue [3]). These variants emerge at different rates due to varying potencies and genetic barriers of resistance of the drugs as well as the ability of the drug-resistant virus to replicate compared with wild type (replication capacity). In HIV-coinfected individuals, drug-resistant variants have not been extensively studied in controlled trials; thus, our knowledge is from a few cohort studies and individual case reports.

Lamivudine has been used most extensively because it was one of the first drugs available to treat HIV infection. Individuals who received lamivudine prior to the approval of tenofovir disoproxil fumarate (TDF) in 2001 are at greatest risk for having lamivudine-resistant HBV because lamivudine was the only drug active against HBV in HIV regimens. Benhamou et al. [4]
demonstrated that in an HIV–HBV-coinfected cohort, 53% had lamivudine-resistant HBV after 2 years of lamivudine monotherapy, which is higher than the 38% in HBV-monoinfected individuals [5]. He also estimated that after 4 years of therapy, 90% would have lamivudine-resistant HBV, which is higher than 67% in HBV-monoinfected individuals. Matthews et al. [6] confirmed this rapid development of lamivudine-resistant HBV in the setting of HIV coinfection. They studied 53 HIV–HBV-coinfected individuals on lamivudine monotherapy for >6 months and found the prevalence of lamivudine-resistant HBV to be 50% in those with <24 months of lamivudine and 94% in those with >48 months of lamivudine monotherapy (Figure 1). This study also demonstrated that at each time point the majority had a single (rtM204V/I) or double mutant (rtM204V+rtL180M) and the minority had the triple mutant (rtV173L+rtL180M+rtM204V).

However, the overall prevalence of the triple mutant was 17%, which is about double the prevalence in HBV monoinfection [7]. Owing to the overlapping reading frames of the HBV Pol and the HBV envelope genes, the triple mutant leads to the surface mutations sE164D/sI195M. In vitro, these surface mutations behave as a vaccine-escape mutant, so there is concern that this mutant virus could be transmitted to individuals who have been vaccinated against HBV [8]. To date, this has not been documented in humans. However, in chimpanzees vaccinated against HBV and then inoculated with the triple mutant, Kamili et al. [9] found that these chimpanzees developed antibodies to the hepatitis B core antigen and T-cell responses against the HBV Pol suggesting that there might have been some replication of the mutant virus. However, HBV DNA was not detectable in the chimpanzees.

Once drug-resistant viruses have developed on lamivudine, continued lamivudine therapy can lead to accumulation of additional mutations, which could compromise other future therapies. In the study by Matthews et al. [6] discussed above, the authors found that 11% had other potentially significant HBV Pol mutations, including mutations at entecavir resistance sites. One individual had the rtQ215S, which was associated with lamivudine and adefovir resistance in one study [10]. However, a recent in vitro study demonstrated that HBV with this variant was still susceptible to lamivudine and adefovir, but differences between the mutant and wild-type constructs in the EC_{50} could not be excluded [11].

The next most commonly used anti-HBV drug in HIV-coinfected patients is TDF given its potent activity against HIV. However, as TDF has a high genetic barrier to resistance for HBV and few studies have examined the development of resistance in HIV coinfection, little is known about the patterns and frequencies of TDF-resistant HBV. The only study to find any potential TDF-resistant mutations is one comprising 43 HIV–HBV-coinfected patients on TDF and ongoing lamivudine, who had detectable viraemia after a mean of 11.2 months on TDF [12]. About half the patients had wild-type virus and the other half had mutations that conferred lamivudine resistance. In addition, a novel mutation, rtA194T, was found in two patients. In one patient, its emergence coincided with an ALT flare and a 1.5 log copy/ml increase in HBV DNA after 62 weeks of TDF. In the other patient, there were no clinical symptoms and no rise in HBV DNA, which does not meet the current definition of resistance as a 1 log increase in HBV DNA. There are conflicting in vitro data regarding whether this rtA194T mutant is resistant to TDF. In a recent study, Amini-Bavil-Olyaee et al. [13] made replication-competent constructs with the rtA194T and found that it impaired replication, which led to a five- to sixfold decreased susceptibility to TDF. Interestingly, when the basal core promoter mutants A1762T and G1764A or the precore mutants G1896A or C1858T were included in the constructs, the replication capacity of the mutant increased.

There is a case report of an interesting envelope mutant that developed on TDF [14]. The report was of an HIV–HBV-coinfected individual on HIV therapy with TDF and lamivudine who at 29 weeks of therapy was found to have an rtV191I without clinical symptoms or a rise in HBV DNA. However, he was hepatitis B surface antigen (HBsAg) negative because the mutation leads to a truncation of the HBsAg, which deletes the last 44 amino acids. The authors found that this
mutation led to decreased lamivudine susceptibility \textit{in vitro}, but not to decreased TDF susceptibility.

Overall, there have been five studies published in HIV–HBV coinfected examining TDF resistance (Table 1). The duration of follow-up in these studies is short, so TDF resistance is unlikely with the longest one being 104 weeks. The only study to demonstrate any mutations is the one discussed above with the rtA194T.

Adefovir has not been extensively used to treat HBV in HIV-infected individuals because the related drug, TDF, is active against both viruses. One cohort study of 35 HIV–HBV-coinfected individuals with adefovir added to lamivudine-based HAART did not demonstrate any resistance mutations after 144 weeks of adefovir [15]. There is one case report of the rtA181T emerging after 30 months of adefovir [16]. This mutant is interesting because it leads to a stop codon in the overlapping envelope gene, which results in defective secretion of viral particles [17]. \textit{In vitro}, coexpression of this mutant with wild type demonstrates that it has a dominant negative effect on virion secretion.

Entecavir was recently recognized to have anti-HIV activity that can result in emergence of drug-resistant HIV [18,19]; thus, its use in HIV–HBV coinfected is restricted to individuals who are on a suppressive HIV regimen. One study enrolled 68 HIV–HBV-coinfected individuals with HIV RNA<400 copies/ml who were on lamivudine-based HAART for at least 24 weeks with a detectable HBV DNA; thus had lamivudine-resistant HBV [20]. Individuals were randomized into a double-blind study of 1.0 mg of entecavir versus placebo with ongoing lamivudine for 24 weeks. After 24 weeks, open label entecavir was given. Not surprisingly, entecavir led to a significant decrease in HBV DNA; however, the response was less than that seen in HBV-infected individuals without HIV coinfection. At week 48, only 8% of the group who received 48 weeks of entecavir had HBV DNA<30 copies/ml and none in the placebo group. This 8% is lower than what has been reported in HBV monoinfection, which is about 33% [21]; thus, there are a large number of individuals at increased risk for developing entecavir resistance mutations. Of those who received 48 weeks of entecavir, 43 of 47 individuals were sequenced and 5% had entecavir-resistance substitutions. This is similar to what has been reported in HBV monoinfection, but further follow-up is needed to see if resistance develops more rapidly, which has been observed with lamivudine.

One issue that is still not clear is whether drug-resistant mutants are present prior to therapy. In one study of 20 HIV–HBV-coinfected individuals in South Africa, 10 (50%) had the rtM204I prior to therapy [22]. In the 15 HBV-monoinfected individuals, 3 (20%) had lamivudine-resistant HBV. Since this was a small study, additional studies are needed to determine if the prevalence of lamivudine-resistant HBV in therapy-naive individuals is truly this high.

### Clinical sequelae of infection by drug-resistant HBV

In order to slow liver disease progression from HBV infection, effective anti-HBV therapy is needed. However, one complication of drug-resistant HBV is the limitation of treatment options because the structural similarity amongst several of the anti-HBV drugs results in overlapping resistance patterns. Thus, if drug-resistant mutations emerge on one drug, other drugs will have decreased or no susceptibility. For example, the rtM204V/I mutant either by itself or with the rtL180M or rtL173T often develops on lamivudine monotherapy, which leads to resistance to telbivudine and emtricitabine as well as reducing susceptibility to entecavir about 30-fold. Likewise, the mutations that lead to entecavir resistance also lead to resistance to lamivudine, emtricitabine and telbivudine.

Similarly, the adefovir resistance mutation rtN236T is still susceptible to TDF, but clinically these mutant viruses have a decreased response to TDF. In a multicentre study of HBV-monoinfected patients, van Bommel et al. [23] retrospectively studied 127 patients on TDF monotherapy for a median of 20 months (range 6–54) of whom 19% had adefovir-resistant virus. In a Kaplan–Meier analysis, the probability of reaching HBV DNA<400 copies/ml was significantly lower in those with adefovir-resistant virus. Thus, adefovir-resistant HBV is clinically more difficult to treat with TDF than HBV without adefovir-resistant mutations.

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**Table 1. Published studies of TDF-resistant HBV in HIV-coinfected individuals**

<table>
<thead>
<tr>
<th>Individuals, n</th>
<th>Duration of TDF, weeks</th>
<th>Individuals with detectable HBV DNA, n (%)</th>
<th>Individuals with TDF mutations, n</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>31</td>
<td>48</td>
<td>4 (12.9)</td>
<td>0</td>
<td>[27]</td>
</tr>
<tr>
<td>43</td>
<td>45</td>
<td>43 (100)*</td>
<td>2</td>
<td>[12]</td>
</tr>
<tr>
<td>65</td>
<td>52</td>
<td>2 (3.1)</td>
<td>0</td>
<td>[28]</td>
</tr>
<tr>
<td>30</td>
<td>52</td>
<td>11 (36.7)</td>
<td>0</td>
<td>[29]</td>
</tr>
<tr>
<td>28</td>
<td>104</td>
<td>4 (14.3)</td>
<td>0</td>
<td>[30]</td>
</tr>
</tbody>
</table>

*Individuals selected for study based on having a detectable HBV DNA. TDF, tenofovir disoproxil fumarate.
Drug-resistant HBV could result in more liver disease progression because the drug-resistant virus is difficult to treat. In HIV–HBV co-infection there are no long-term studies that directly address this issue. One study from the Multicentre AIDS Cohort Study sheds some light on this. Hoffmann et al. [24] compared 350 Multicentre AIDS Cohort Study participants without markers for a HBV infection with 45 individuals with chronic hepatitis B who initiated HAART. When looking at the outcome of non-AIDS deaths, those with HBV had a rate of 22/1,000 person-years (PYs) compared with 2.4/1,000 PYs in those without HBV markers. The liver-related mortality rate in those with HBV infection was 17/1,000 PYs, which was similar to the liver-mortality rate in the pre-HAART era of 14/1,000 PYs [1]. In the four men who died of liver disease, HBV DNA was tested on three of them and two were detectable with lamivudine-resistant virus. Further studies are needed to determine if those with lamivudine-resistant virus have similar liver mortality rates to those receiving HIV therapy that includes more potent anti-HBV agents, such as TDF.

Although development of mutant virus usually occurs in the context of ALT flares, in HIV-infected individuals flares might not occur. Bhattacharya et al. [25] looked at the ALT values and the presence of drug-resistant virus in 45 different samples from 11 patients. In those samples with only wild-type virus, the median ALT activity was 43 IU/ml, whereas in those with drug-resistant variants the median ALT was 44 IU/ml. This lack of flare might be due to a compromised immune system. This study emphasizes the need to monitor with both HBV DNA and ALT for the emergence of drug-resistant virus in HBV-infected patients.

The other potential concern in HIV-infected individuals is that immune reconstitution syndrome could occur with the emergence of a drug-resistant variant, potentially resulting in fulminant hepatic disease. Gouskos et al. [26] reported a case of severe and prolonged HBV-specific CD8+ T-cell response associated with emergence of a lamivudine-resistant HBV that resulted in a severe flare of liver disease with ALT>1,000 IU/l.

Conclusions

HBV that is resistant to the 1-nucleosides is common in HIV-infected individuals because of the more rapid emergence of drug-resistant virus and the long-standing use of lamivudine monotherapy in the early years of effective antiretroviral therapy. If lamivudine-resistant HBV is present, continued therapy with lamivudine leads to emergence of additional mutations that can compromise use of other anti-HBV agents. Whether drug resistance occurs more rapidly in HIV infection to some of the newer agents is not known. Owing to the overlapping polymerase and envelope reading frames, drug-resistant variants can lead to surface antigen negative mutants. Clinical monitoring of ALT and HBV DNA is necessary to detect emergence of drug-resistant variants. Immune reconstitution syndrome to drug-resistant variants might also occur.

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Disclosure statement

The author declares no competing interests.

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