Managing Drug Resistance in HBV

Yogesh Chawla, Bhupesh Singla, Balkrishan Sharma

INTRODUCTION

Chronic hepatitis B virus (CHB) infection is a major cause of severe liver related morbidity and premature mortality. The main goal of treatment for HBV infection is to achieve sustained suppression of viral replication, which reduces hepatic necroinflammation, the development of fibrosis, and disease progression. Interferon-α, the first available agent for the treatment of HBV infection, acts by inhibiting viral entry into hepatocytes and activate viral ribonuclease to inhibit HBV replication. However, it is primarily an immunomodulating agent.1 With the advent of oral nucleoside analog therapy, the management of CHB infection has improved substantially in the last decade. These agents are well tolerated, very effective at suppressing viral replication, and appear to be safe. A major shortcoming, however, of nucleoside analog therapy is the high rate of virological relapse when treatment is discontinued. Therefore, treatment must often be administered long-term, if not indefinitely. The emergence of drug resistance during long-term monotherapy with nucleos(t)ide analogues is almost inevitable and represents a clinical challenge as it frequently negates the benefits of therapy and sometimes may be associated with hepatitis flares and even death. As was the case with the human immunodeficiency virus (HIV), the management of HBV infection is dynamic and subject to change as new information becomes available.

AIM OF TREATMENT

The major goals of antiviral therapy include (i) the achievement of HBeAg seroconversion, (ii) undetectability of HBV DNA levels by real time polymerase chain reaction (iii) persistent normalization of serum aminotransferase (ALT) levels. HBsAg loss and seroconversion to anti-HBs is another long-term goal of treatment which is difficult to achieve. Ideally, patients should become HBV DNA undetectable during therapy, because persistent viraemia has been associated with a higher rate of viral resistance. Long-term goals such as improved survival, prevention of cirrhosis and disease complications are even more important and are achievable if treatment results in a durable virological response with the lowest level of HBV DNA possible.2

Viral Replication and Selection of Resistant Strains

The HBV consists a small genome of 3.2-kb, which is partially double stranded and comprises four overlapping open reading frames (ORFs).3 The viral genome is transcribed into four major subgenomic viral messenger RNAs, under the control of specific enhancers and is the template for the pregenomic RNA.4 The virus is encapsidated after binding of the polymerase and core to the pregenomic RNA in the cytoplasm. Nucleocapsids are enveloped by budding into the endoplasmic reticulum, after which they are secreted from the cell or return to the nucleus to amplify the cccDNA reservoir.5 Viral mutations occur spontaneously during HBV replication as viral reverse transcriptase lack proof reading capacity. These replication errors result in the emergence of multiple HBV variant quasispecies that coexist and reach population densities in direct proportion to their relative replication fitness.3 This phenomenon is responsible for the generation of significant diversity; it has been shown that HBV genomes in one given patient displayed a rate of 1.4 to 3.2x10⁻⁵ nucleoside substitutions per year, a value approximately 10⁴ times greater than DNA genomes.6 A
chronic HBV carrier can produce up to 10^13 virions per day, and as a result, every nucleotide of the 3.2-kb HBV genome theoretically can be substituted within one patient every day.  

Usually mutant viruses are less fit, meaning they do not replicate at the same rate as wild-type virus, but may have a survival advantage in the presence of an antiviral agent. Over time, compensatory or secondary mutations develop after the initial mutation, which restores functional defects in the viral polymerase caused by the primary mutations. These compensatory mutations enable the mutant virus to replicate at near wild-type levels, leading to the development of antiviral resistance.

The potency of a nucleoside analog acting as an antiviral agent is determined by its ability to serve as a competitive inhibitor of the HBV polymerase relative to that of the natural substrate. Clinically, potency of a drug is reflected by how rapidly it can suppress viral replication; if more rapid, then lower the risk of developing antiviral resistance.

An antiviral agent is said to have modest antiviral activity if it has high genetic barrier to resistance. The genetic barrier refers to the number of mutations required by virus in order to replicate efficiently in the presence of the antiviral agent. The genetic barrier to resistance is partly dependent on the structure of the antiviral compound and the constraints imposed by the ability of the viral polymerase to tolerate compensatory mutations without significantly impairing its enzymatic activity. A number of other host factors indirectly contribute to the development of antiviral resistance. Immune suppression, whether innate or acquired, can affect the rate of viral replication and, therefore, the rate of development of mutations. Finally, existing mutations due to prior use of antiviral agents can lower the genetic barrier to resistance of some drugs.

CLINICAL ASPECTS OF RESISTANCE

Definitions

Defining which nucleotide changes confer drug resistance and how new mutations are characterized will allow for the true incidence of antiviral resistance to be determined as well as permit direct comparison of the available antiviral agents.

Primary Nonresponse. Primary nonresponse is defined as the inability of the antiviral agent to reduce serum HBV DNA by ≥1 log_{10} IU/mL within the first 6 months of treatment. Primary nonresponse is related to the potency of the antiviral agent and perhaps to polymorphisms of host enzymes that are involved in converting prodrugs to their active moiety. There is no evidence that pre-existing antiviral mutations are associated with primary nonresponse. The observation of primary nonresponse is an indication to change therapy as it may increase risk for the development of resistance.

Virological Breakthrough. Virological breakthrough is the first clinical indication of the development of antiviral drug resistance. It is defined as an increase in serum HBV DNA by ≥1 log_{10} IU/mL above a nadir on two or more consecutive occasions at least 1 month apart while on treatment after achieving an initial response in a compliant patient.

Biochemical Breakthrough. It is defined as an elevation in serum ALT level while on treatment after achieving normalization in a compliant patient. Biochemical breakthrough usually lags behind virological breakthrough, and serum ALT levels may remain normal for weeks to years after the development of antiviral resistance. Thus, serum ALT is not a sensitive indicator of antiviral resistance.

Genotypic Resistance. Genotypic resistance refers to the detection of viral populations bearing amino acid substitutions in the reverse transcriptase region of the HBV genome that have been shown to confer resistance to antiviral drugs in a phenotypic assay during antiviral therapy.

Main sites of drug resistance in HBV

Currently, lamivudine, adefovir, entecavir, telbivudine and tenofovir have been approved worldwide after large-scale randomized controlled trials in hundreds or thousands of

![Fig.1: Kinetics of drug resistance emergence](image-url)
patients. These developments have led to better management of chronic HBV infection and also raised the hope of long-term success in the prevention of disease progression. The major loci responsible for resistance to available HBV antivirals i.e lamivudine (LMV), telbivudine (LdT), adefovir (ADV), entecavir (ETV) and tenofovir (TDF) are given in Fig.2.

Polymerase protein of HBV is divided into four parts namely terminal protein, spacer, reverse transcriptase (RT) and RNaseH. Drug resistance occurs because of mutations in RT region of polymerase protein.

Incidence of oral antiviral resistance

**Lamivudine**: LMV resistance increases progressively over the course of treatment; 14%-32% of patients become resistant to the drug each year after treatment was initiated, and more than 80% are resistant after 48 months of treatment.20

**Entecavir**: Virologic breakthrough is rare in nucleoside-naive patients, and is observed only in 3.6% of patients by 96 week of entecavir treatment.21 Preliminary data suggest that the rate of entecavir resistance remained at 1.2% in nucleoside-naive patients, after 5 years of treatment.22

**Telbivudine**: Telbivudine selects for mutations in the YMDD motif. The resistance rate is substantial and increases exponentially after the first year of therapy. In the phase III clinical trial, genotypic resistance after 1 and 2 years of treatment was observed in 5.0% and 25.1% of HBeAg positive and in 2.3% and 10.8% of HBeAg-negative patients who received Telbivudine in the phase III clinical trial.14

**Adefovir**: Rate of resistance is slower during adefovir treatment compared to Lamivudine and no adefovir-resistant mutations were found after 1 year of treatment in the patients who participated in the Phase III trials.23 Cumulative rate of genotypic resistance to adefovir in the phase III trial in HBeAg-positive patients was estimated to be 20% after 5 years of treatment.24

**Tenofovir**: Only one study has reported alanine to threonine substitution at position 194 (rtA194T) as a mutation associated with resistance to tenofovir in two patients with HBV and HIV coinfection.25 Subsequent studies have not confirmed the same mutation. A recent study found that the rtA194T mutation is associated with decreased replication fitness in in vitro studies but replication can be restored in the presence of precore G1896A stop codon mutation.26

Managing of Antiviral Resistance

**Lamivudine**

The management options for patients with Lamivudine resistance are evolving and are based on available data from clinical trials and *in vitro* testing. Therapeutic options include adding adefovir, switching to drugs with higher potency and higher genetic barriers to resistance such as entecavir or tenofovir.27,28,29 (Table I) Emerging data suggests

![Primary antiviral drug resistance mutations](image-url)
that entecavir is associated with an unacceptably high rate of resistance in patients with lamivudine resistance. For patients who are HBeAg-positive, rates of undetectable HBV DNA at week 48 in those who were switched to entecavir 1.0 mg daily or tenofovir 300 mg daily were 35%, 21%, and 91%, respectively. For patients who are HBeAg-negative with Lamivudine resistance, the virological response to salvage therapy is better compared to patients who are HBeAg-positive with resistance, as is the case in treatment-naïve patients. In one study, 66% and 64% of patients treated by switching or adding on adefovir therapy, respectively, achieved a virological response.

Adefovir
For the management of adefovir resistance, evidence is based on small case reports and in vitro testing. For patients with the rtN236T mutation, options include switching to or adding entecavir, adding lamivudine, switching to tenofovir. In case the rtA181T mutation has developed, options are fewer and include switching to or adding entecavir, or switching to tenofovir. Lamivudine should not be used in this setting because of risk of cross-resistance with the rtA181T mutation.

Entecavir
Management of entecavir resistance is based on data derived largely from case reports and in vitro phenotypic testing. On the basis of this limited evidence, three approaches are available: to switch or add adefovir; or to switch or add tenofovir; or switch to tenofovir plus emtricitabine (off-label use).

Telbivudine
There is limited information on management of resistance to telbivudine; however, the recommendations would be similar to those for lamivudine resistance based on the similarities of these two compounds. American Association for the Study of Liver Diseases (AASLD) recommendations for management of drug resistance in chronic hepatitis B is given in table II.

MULTIDRUG RESISTANCE
Scant data is available on the management of multidrug-resistant HBV. Every precaution should be taken to prevent this clinical scenario from occurring because of limited management options left when it occurs. Peginterferon may have a role in carefully selected cases and expert advice should be sought in managing these patients. Although most HBV strains that are resistant to a particular nucleoside analogue can be effectively suppressed by a nucleoside analogue from a different structural group, multidrug resistance is a major problem, especially in the context of prolonged and sequential antiviral treatment. The risk of emergence of multidrug resistance is directly related to inadequate treatment as a result of partial viral suppression. Multidrug resistance can also occur in patients who received drugs with a similar cross-resistance profile during different times of treatment. To avoid the development of multidrug-resistant HBV, there should be efforts to target maximum viral suppression with a selection of drugs that have complementary cross-resistance profiles.

Table I. Primary Resistance Mutations, Frequency of Resistance at 1 Year and Preferred Management Strategies to Resistance with the Approved Nucleoside Analogs.

<table>
<thead>
<tr>
<th>Antiviral</th>
<th>Primary drug resistance mutation</th>
<th>HBeAg +ve Freq. of resistance in 1 year</th>
<th>HBeAg -ve Freq. of resistance in 1 year</th>
<th>Preferred Management</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lamivudine</td>
<td>rtM204V/I rtA181V/T</td>
<td>11-32%</td>
<td>11-27%</td>
<td>Add adefovir or switch to tenofovir</td>
</tr>
<tr>
<td>Telbivudine</td>
<td>rtM204I</td>
<td>5%</td>
<td>2%</td>
<td>Add adefovir or switch to tenofovir</td>
</tr>
<tr>
<td>Adefovir</td>
<td>rtA181V/T rtN236T</td>
<td>0%</td>
<td>0%</td>
<td>Add lamivudine or Telbivudine or Switch to tenofovir or Switch to entecavir</td>
</tr>
<tr>
<td>Tenofovir</td>
<td>None</td>
<td>0%</td>
<td>0%</td>
<td>?</td>
</tr>
<tr>
<td>Entecavir</td>
<td>rTL180M &amp; rTM204M &amp; rT1169T and rTM205V &amp; rTL184G and rT5201</td>
<td>0%</td>
<td>0%</td>
<td>Add adefovir or switch to tenofovir</td>
</tr>
</tbody>
</table>
Prevention
- Avoid unnecessary treatment
- Initiate treatment with potent antiviral that has low rate of drug resistance or with combination therapy
- Switch to alternative therapy in patients with primary non-response

Monitoring
- Test for serum HBV DNA (PCR assay) every 3-6 months during treatment
- Check for medication compliance in patients with virologic breakthrough
- Confirm antiviral resistance with genotypic testing

Treatment

<table>
<thead>
<tr>
<th>Resistance Type</th>
<th>Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lamivudine-resistance</td>
<td>Add adefovir or tenofovir</td>
</tr>
<tr>
<td></td>
<td>Stop lamivudine, switch to Truvada **</td>
</tr>
<tr>
<td>Adefovir-resistance</td>
<td>Add lamivudine#</td>
</tr>
<tr>
<td></td>
<td>Switch to or add entecavir##</td>
</tr>
<tr>
<td>Entecavir-resistance</td>
<td>Switch to tenofovir or Truvada</td>
</tr>
<tr>
<td>Telbivudine-resistance</td>
<td>Add adefovir or tenofovir</td>
</tr>
<tr>
<td></td>
<td>Stop telbivudine, switch to Truvada</td>
</tr>
</tbody>
</table>

CONCLUSIONS

Nucleoside and nucleotide analogues are potent HBV suppressors, but these agents rarely eradicate HBV. Therefore, the durability of viral response is a problem, and long-term therapy is usually required to ensure maintained HBV suppression. However, preventing and managing antiviral resistance is a critical challenge during long-term nucleos(t)ide analogue maintenance therapy. The emergence of these mutants is characterized by an increasing level of serum HBV DNA, elevation of ALT level, and even hepatitis flare or decompensation. The prevention and proper management of drug resistance are crucial to ensure long-term success, the most effective way to achieve this is not clear. To start treatment in the right patients at the right time with the right drug is essential in minimizing the problem of drug resistance. Each of these agents has a different profile of resistant mutations. In choosing a direct antiviral agent to initiate therapy, resistance profile is a crucial factor to consider, apart from potency and cost. In the case of drug resistance emerging, timely institution of a drug without cross resistance may rescue the adverse effects of drug resistance and ensure the long-term success of nucleos(t)ide analogue therapy. To develop strategies for enhancing the therapeutic response and shortening the duration of therapy is an ultimate goal to avoid the problems of drug resistance.

REFERENCES

hepatitis B in nucleoside-naive patients is not due to pre-existing drug-resistant mutants. Antivir Ther 2008;13:381-388.


