LIVER TRANSPLANTATION 14:S8-S14, 2008

How To Diagnose and Treat Hepatitis B Virus Antiviral Drug Resistance in the Liver Transplant Setting

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Received May 8, 2008; accepted July 21, 2008.

Key Points

1. Hepatitis B virus variants with antiviral drug–resistant mutations and/or hepatitis B immune globulin–resistant mutations are the main cause of hepatitis B virus reinfections post–liver transplant.
2. Early diagnosis of antiviral drug resistance and prompt initiation of rescue therapy are important in preventing hepatitis flares and hepatic decompensation.
3. Virologic breakthrough is the first indication of antiviral drug resistance.
4. Genotypic resistance testing should be performed when possible to avoid unnecessary modification of treatment in patients who do not have confirmed antiviral drug resistance and to permit appropriate selection of rescue therapy in those who have confirmed antiviral drug resistance.
5. Choice of rescue therapy requires knowledge of the past history of hepatitis B virus treatments and virologic response to those treatments, patterns of mutations detected at the time of virologic breakthrough, and in vitro cross-resistance data.
6. Occurrence of antiviral drug resistance can be reduced by the use of the most potent nucleos(t)ide analogue(s) with the highest genetic barrier to resistance, emphasis of medication compliance, and close monitoring of virologic response. ***Liver Transpl 14:S8-S14, 2008.***

The availability of orally administered nucleos(t)ide analogues that are safe and effective in suppressing hepatitis B virus (HBV) replication in pre– and post–liver transplant patients has revolutionized the management of these patients. However, these therapies do not eradicate HBV. Therefore, most patients require long-term treatment to maintain virus suppression. Life-long treatment is generally recommended for liver transplant patients to prevent hepatitis flares associated with virologic relapse because these flares may lead to rapid decompensation and death in patients who have limited hepatic reserve or are immunosuppressed, but the risk of antiviral drug resistance increases with the duration of treatment. Thus, while the use of nucleos(t)ide analogues pre-transplant and combination of nucleos(t)ide analogues and hepatitis B immune globulin (HBIG) post-transplant have been shown to reduce the rate of HBV reinfection post-transplant to less than 10%, almost all cases of HBV reinfection are due to HBV variants with antiviral drug–resistant mutations and/or HBIG-resistant mutations.[1-3] This review discusses the nomenclature, diagnosis, treatment, and prevention of HBV antiviral drug resistance.

NOMENCLATURE

Antiviral drug resistance may be reported as phenotypic resistance, genotypic resistance, viral resistance, or clinical resistance. Table 1 summarizes the definitions of the most common terms used to describe antiviral drug resistance.[4] *Phenotypic resistance* is defined as decreased susceptibility (ie, increased concentrations of the drug are needed to achieve 50% or 90% inhibition) of an HBV polymerase to an antiviral drug in *in vitro* assays. *Genotypic resistance* is defined as the detection during antiviral therapy of viral populations

Abbreviations: HBIG, hepatitis B immune globulin; HBV, hepatitis B virus; MALDI-TOF, matrix-assisted laser desorption/ionization time-of-flight; RFLP, restriction fragment length polymorphism.

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DOI 10.1002/lt.21616
Published online in Wiley InterScience (www.interscience.wiley.com).

TABLE 1. Nomenclature for Antiviral Resistance

Term	Definition
Virologic breakthrough	Increase in serum HBV DNA by >1 $\log_{10}$ above nadir while patient is on treatment after achieving initial response
Biochemical breakthrough	Increase in serum alanine aminotransferase while patient is on treatment after achieving initial response
Genotypic resistance	Detection of amino acid substitutions in the reverse transcriptase region of the HBV polymerase gene that has been shown to decrease susceptibility to treatment
Phenotypic resistance	In vitro confirmation that the mutation decreases susceptibility to treatment

Abbreviation: HBV, hepatitis B virus.

bearing amino acid substitutions in the reverse transcriptase region of the HBV polymerase gene that have been shown to confer resistance to antiviral drugs in phenotypic assays. *Viral resistance* or *virologic breakthrough* is defined as an increase in serum HBV DNA by at least 1 $\log_{10}$ (10-fold) above nadir or the reappearance of serum HBV DNA in the patient with previously undetectable HBV DNA on ≥2 occasions at least 1 month apart while the patient is on treatment and after initial response is achieved in the medication-compliant patient. The term *clinical resistance* is often used synonymously with *biochemical breakthrough* and is defined as an elevation in serum alanine aminotransferase while the patient is on treatment and after normalization is achieved in the medication-compliant patient. Ascertainment of medication compliance is problematic because assays for monitoring nucleos(t)ide drug concentrations are not readily available and self-reporting by patients may not be reliable. As many as 30% to 50% of virologic breakthroughs observed in clinical trials are attributed to noncompliance with medications[5-8]; this figure is likely higher in clinical practice.

DIAGNOSIS

Genotypic resistance may precede viral resistance (virologic breakthrough) by many months, and viral resistance may precede clinical resistance (biochemical breakthrough) by months to years. Because of the potential devastating effect of biochemical breakthrough in liver transplant patients, early diagnosis of antiviral drug resistance and prompt intervention are critical. Thus, confirmation of virologic breakthrough on repeat testing is not necessary if there is a marked increase in serum HBV DNA or if there is accompanying biochemical breakthrough.

Detection of virologic breakthrough requires knowledge of the pretreatment serum HBV DNA level, documentation of initial response, determination of nadir or lowest HBV DNA level achieved, and detection of a ≥1 $\log_{10}$ increase (or reappearance) in the HBV DNA level. Therefore, establishment of the baseline serum HBV DNA level and regular monitoring, preferably at 3-month intervals with the same HBV DNA assay at each measure, are necessary for the diagnosis of viral resistance. Without documentation of initial response, it is not possible to determine if a patient who has a high serum HBV DNA level after a long duration (≥6 months) of nucleos(t)ide analogue therapy has never achieved adequate viral suppression or instead has virologic breakthrough. This is particularly problematic for patients receiving adefovir, which has weak antiviral activity at the approved dose.

Because not all breakthroughs are due to drug resistance, confirmation with genotypic resistance testing should be performed whenever possible. This will avoid unnecessary modification of treatment in patients who do not have confirmed antiviral drug resistance and permit appropriate selection of rescue therapy in those who have confirmed antiviral drug resistance. Genotypic resistance testing is particularly important in guiding the choice of rescue therapy for patients who have been exposed to more than 1 nucleos(t)ide analogue as multidrug resistance mutants have been reported in patients who have received sequential monotherapies.[9-11]

A variety of assays have been used for the detection of HBV antiviral drug–resistant mutations (Table 2). Direct (population) sequencing is the least sensitive method for detecting minor populations of drug resistance mutants. This method requires that the mutant population composes approximately 20% of the total HBV quasispecies pool for detection. However, direct sequencing allows all mutations to be identified, and this is important when we are evaluating resistance to new therapies for which the full spectrum of resistant mutations has not been determined and when we are evaluating drug resistance in patients who have received multiple nucleos(t)ide analogues. Other assays such as restriction fragment length polymorphism and reverse hybridization (line probe) assays can detect viral mutants that constitute as little as 5% of the total viral population.[12,13] Because these assays are more sensitive, they enable earlier identification of patients with genotypic resistance[14,15] and may be particularly useful in the management of liver transplantation patients. A major disadvantage of these assays is that they

TABLE 2. Assays Used To Detect Antiviral Drug–Resistant Hepatitis B Virus Mutations

Assay	Advantages	Disadvantages
Direct sequencing	Detects all mutations Most useful with new therapies	Least sensitive at detecting minor populations (~20%)
Sequencing of multiple clones	Detects all mutations Sensitivity depends on number of clones sequenced.	Labor-intensive
RFLP, line probe	Sensitive (~5%) Early detection of genotypic resistance	Detects only known mutations
Single genome sequencing, MALDI-TOF mass spectrometry	Ultrasensitive (~0.1%)	Cannot differentiate spontaneous mutations from mutations selected during treatment

Abbreviations: MALDI-TOF, matrix-assisted laser desorption/ionization time-of-flight; RFLP, restriction fragment length polymorphism.

TABLE 3. Signature Mutations Associated with Antiviral Resistance

Nucleos(t)ide Analogue	Mutation	Comment
Lamivudine	M204V/I	Resistance
	A181T	Resistance
	L80, V173L, L180M	Compensatory
Telbivudine	M204I	Resistance
	L80, L180M	Compensatory
Adefovir	A181V or N236T	Resistance
	A181T	Resistance
	I 233V	Primary nonresponse*
Entecavir	T184S/A/I/L/F/G/M/C	Resistance†
	S202G/C	Resistance†
	M250V/L/I	Resistance†
Tenofovir	A194T	Resistance*

*Initial report of resistance not confirmed.
†In association with M204V/I.

can detect only known mutations, and existing assays may not detect all mutations that have been shown to be associated with resistance to HBV antiviral drugs. Recently, several ultrasensitive assays, such as single genome sequencing and mass spectrometry–based restriction fragment mass polymorphism, which can detect mutants that constitute <0.1% of the viral quasispecies, have been used to detect antiviral drug–resistant HBV mutations,[16,17] but the utility of these ultrasensitive assays in predicting viral resistance and the need for modification of treatment has not been established.

MUTATIONS ASSOCIATED WITH RESISTANCE TO NUCLEOS(T)IDE ANALOGUE THERAPIES

Mutations associated with antiviral resistance may be classified as primary (being responsible for decreased susceptibility to the drug) or compensatory (being responsible for restoring replication fitness of the mutant virus). Signature mutations associated with resistance to the approved HBV therapies have been characterized (Table 3).[4] As an increasing number of patients with divergent HBV sequences are exposed to nucleos(t)ide analogue therapies, additional mutations may be identified. However, caution must be exercised in attributing differences in HBV sequences as the cause of antiviral drug resistance, particularly when baseline sequences are not available for comparison. Thus, the significance of several mutations such as alanine-to-threonine substitution at position 181 (rtA181T) and resistance to adefovir, isoleucine-to-valine substitution at position 233 (rtI233V) and primary nonresponse to adefovir, and alanine-to-threonine substitution at position 194 (rtA194T) and resistance to tenofovir remains controversial.[18-21]

Among nucleoside-naïve patients, antiviral drug resistance has been reported in up to 70% of patients after 4 years of lamivudine, 29% after 5 years of adefo-

TABLE 4. Treatment Options for Patients with Antiviral Resistance

Type of Resistance	Rescue Therapy
Lamivudine or telbivudine resistance	Add adefovir or tenofovir. Switch to emtricitabine and tenofovir.
Adefovir resistance*	Add lamivudine or switch to emtricitabine and tenofovir. Add entecavir.
Entecavir resistance*	Add adefovir or tenofovir.
Multidrug resistance	?

* Limited in vivo data.

vir, 1% after 5 years of entecavir, and 9% to 22% after 2 years of telbivudine.[22-25] Resistance rates are substantially higher in patients with prior resistance to lamivudine, with rates of up to 20% after 2 years of switching to adefovir monotherapy and 51% after 5 years of switching to entecavir.[25,26]

TREATMENT OF PATIENTS WITH ANTIVIRAL DRUG–RESISTANT HBV

Optimal management of patients with antiviral drug–resistant HBV requires knowledge of the past history of HBV treatments and virologic response to those treatments, patterns of mutations detected at the time of virologic breakthrough, and in vitro cross-resistance data. Rescue therapy should be initiated early when virologic breakthrough is confirmed to be due to drug resistance.[27] If genotypic resistance testing is not readily available, rescue therapy should be initiated in patients who have virologic breakthrough despite medication compliance. Options for rescue therapy against antiviral drug–resistant HBV are listed in Table 4.[4,28]

Lamivudine and telbivudine resistance

Lamivudine and telbivudine are L-nucleosides that select for mutations at the same site: methinone to valine or isoleucine at position 204 (rtM204V/I) for lamivudine and only rtM204I for telbivudine. In vitro studies demonstrated that adefovir, tenofovir, and entecavir have antiviral activity against rtM204V/I. The antiviral activity of adefovir against rtM204V/I is comparable to its activity against wild-type HBV, but its activity against rtA181T appears to be lower.[29,30] A pilot study in patients with lamivudine-resistant HBV reported that adefovir monotherapy resulted in similar rates of viral suppression when compared with combination therapy of lamivudine and adefovir.[31] However, the combination of lamivudine plus adefovir has been shown to be more effective in preventing adefovir resistance than adefovir monotherapy.[32,33]

In a study of 467 pretransplant and posttransplant patients who received additional adefovir for lamivudine-resistant HBV, serum HBV DNA became undetectable in 59% of wait-listed patients and 40% of posttransplant patients after 48 weeks of treatment.[34] Nephrotoxicity, defined as a confirmed increase in serum creatinine by ≥0.5 mg/dL from baseline, was observed in 6% of wait-listed patients, 47% of patients who underwent liver transplantation during the course of the study, and 21% of posttransplant patients. Many patients were receiving concomitant nephrotoxic medications, and some developed hepatorenal syndrome during the course of the study; thus, it is difficult to determine the role of adefovir in the worsening of renal function in these patients. In this study, only 9 patients had a confirmed decrease in serum phosphorus to <2 mg/dL. Serum creatinine should be closely monitored in all liver transplant patients receiving adefovir (and tenofovir), and dose adjustments should be made according to renal function.

Tenofovir has also been shown to be effective in suppressing lamivudine-resistant HBV in clinical studies.[35,36] At the approved dose of 300 mg daily, tenofovir has more potent antiviral activity than 10 mg of adefovir and a similar safety profile.[37-40]

The activity of entecavir against lamivudine-resistant HBV is significantly lower than its activity against wild-type HBV, and the presence of an rtM204V/I mutation decreases the genetic barrier to entecavir resistance.[41] Clinical studies showed that despite the use of a higher dose (1.0 versus 0.5 mg/day in patients without lamivudine resistance), a 48-week course of entecavir suppressed serum HBV DNA to undetectable levels in a smaller percentage of hepatitis B e antigen–positive lamivudine-refractory patients (19% versus 67%), and continued treatment for up to 5 years resulted in a higher rate of entecavir resistance (50% versus 1% after 5 years of treatment) in comparison with nucleos(t)ide naïve patients.[25,42]

The best approach for patients with lamivudine resistance is to continue lamivudine and add adefovir. In patients with suboptimal viral suppression and those with the rtA181T mutation, tenofovir may be substituted for adefovir. Tenofovir should replace adefovir when it is approved for HBV treatment because it has more potent antiviral activity. Entecavir is not an optimal treatment for lamivudine-refractory patients because of the high risk of subsequent entecavir resistance. This same approach can be applied to patients with telbivudine resistance.

Adefovir resistance

In vitro studies have demonstrated that lamivudine, telbivudine, and entecavir all have antiviral activity

against adefovir-resistant mutations, but the activity of lamivudine (and likely telbivudine) against the rtA181V/T mutation is lower in comparison with wild-type HBV.[43]

Clinical data regarding rescue therapy for patients with adefovir-resistant HBV are limited. Case reports have suggested that lamivudine is effective in suppressing serum HBV DNA levels in patients with adefovir resistance.[44,45] However, the durability of response, particularly in patients with previous lamivudine resistance, is unknown. Furthermore, re-emergence of lamivudine-resistant mutations in this population has been observed within a few months of the reintroduction of lamivudine.[11] Tenofovir has been reported to result in decreases in serum HBV DNA levels in patients with adefovir-resistant HBV, possibly because of the higher dose used: 300 mg versus 10 mg for adefovir. However, the efficacy of tenofovir monotherapy in this setting is limited because of cross-resistance between tenofovir and adefovir.[46,47] Entecavir has been shown in case reports to be effective in suppressing adefovir-resistant HBV.[26]

The best approach to treating patients with adefovir resistance is to add lamivudine, telbivudine, or entecavir (Table 4). Entecavir may be a better option for patients with previous lamivudine resistance and in patients with the rtA181V/T mutation.

Entecavir resistance

Resistance to entecavir occurs through a 2-hit mechanism.[41] Initially, HBV with an rtM204V/I mutation is selected because these mutants are less sensitive to entecavir in comparison with wild-type HBV. Virologic breakthrough occurs only after the emergence of additional entecavir resistance mutations at codons 184, 202, and 250. Entecavir resistance mutations are sensitive to adefovir and tenofovir in vitro, but in vivo data supporting their efficacy are based on isolated case reports only.[26,48,49]

The only option for treating patients with entecavir resistance is to add adefovir or tenofovir (Table 4).

HBIG resistance

Mutations in the hepatitis B surface protein have been detected in patients who developed HBV reinfection after liver transplantation despite HBIG prophylaxis. The most common mutation involves glycine-to-arginine substitution at position 145 (sG145R).[50] Lamivudine has been shown to be effective in suppressing serum HBV DNA levels in posttransplant patients who received HBIG prophylaxis only and who developed recurrent hepatitis B.[51,52] It is likely that other HBV nucleos(t)ide analogues have similar efficacy.

Multidrug resistance

Sequential treatment with nucleos(t)ide analogue monotherapy has resulted in the sequential selection of mutations conferring resistance to both the initial and subsequent rescue therapy.[10,11] Combinations of mutations to antiviral drugs and HBIG have also been reported in liver transplant recipients who received antiviral therapy and HBIG prophylaxis.[9] The best approach to treating patients with multidrug resistance is unclear. Careful characterization of the pattern of mutations is needed to guide the choice of rescue therapy in these patients.

CONCLUSIONS

Antiviral drug resistance is the most important cause of HBV reinfection after liver transplantation; therefore, every effort must be made to prevent its occurrence. Initial therapy should use the most potent nucleos(t)ide analogue with the highest genetic barrier to resistance, and the importance of medication compliance should be emphasized. Response should be closely monitored, and modification of treatment should be considered in patients who fail to achieve rapid virus suppression. Combination therapy with another drug that is not cross-resistant should be used if antiviral drugs with a low genetic barrier to resistance such as lamivudine or telbivudine or drugs with weak antiviral activity such as adefovir are used in liver transplant patients. Whether de novo combination therapy offers any advantage compared to monotherapy with potent drugs that have a high genetic barrier to resistance remains to be tested.

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