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Resistance to antiviral in chronic hepatitis B therapy – clinical implications

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Summary

The problem of resistance in chronic hepatitis B treatment has increased from the time when the first nucleoside analog was used. The authors try to explain this phenomenon. Approved antivirals (lamivudine, entecavir, adefovir) and appearance of HBV resistant to these drugs are described in this article. Some therapeutic aspects and possibilities in treating patients resistant to nucleoside analogs are presented.

key words: **chronic hepatitis B • resistance • antiviral treatment**

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BACKGROUND

Antiviral therapy for chronic hepatitis B (CHB) remains a clinical challenge. Eradication of HBV in case of chronic hepatitis B is probably impossible. However, even in absence of seroconversion, antiviral therapy could aim to achieve long-term suppression of viral load, witch has been proven to translate into a remission of liver disease. There are currently 5 approved therapies for the treatment of chronic HBV infection: standard interferon, pegylated interferon, lamivudine, adefovir and entecavir. During interferon therapy, some adverse events can occur while long-term antiviral treatment can lead to development of resistance. Unfortunately, in the management of chronic hepatitis B, the nucleoside analogs (NA) have essentially similar mechanisms and sites of action. The frequency of detection of antiviral-resistant HBV mutants correlates with pretreatment serum HBV DNA level, rapidity of viral suppression, and duration of nucleoside analog treatment.

DEFINITION OF RESISTANCE

Resistance occurs sequentially at three levels. At the first level, so-called genotypic resistance, mutations in the HBV genome that are known to confer resistance are detected during antiviral therapy. This is followed by virological breakthrough, which is a rebound in serum HBV-DNA following the development of genotypic resistance. The last step is clinical breakthrough, with increased serum alanine aminotransferase (ALT) levels and worsening of liver histology [1].

An antiviral effect is defined as a minimum reduction in serum HBV DNA of 1 log10 IU/mL from the pre-treatment baseline within the first 3 months. Failure to achieve this decrease constitutes *primary treatment failure*. A confirmed increase in serum HBV DNA of 1 log10 IU/mL from the nadir following initially effective treatment constitutes *secondary treatment failure*. Genotyping and/or phenotyping of clinical isolates are required to confirm that treatment failure is due to resistant virus [1].

The viral replication fitness is an important determinant of disease progression for CHB. In general, mutations that confer resistance to NA reduce replication fitness, at least in vitro, but secondary compensatory mutations that restore replication fitness are common and may also modify drug resistance phenotypes [2].

VIRAL RESISTANCE

Hepatitis B virus (HBV) is a human DNA virus, which replicates via reverse transcription phase when the viral polymerase has a spontaneous error rate that leads to the accumulation of point mutation. As a result, the mutation rate for HBV is higher than the rate observed for most DNA viruses. HBV are classified into genotypes based on genomic sequencing, and antigenic subtypes based on the antigenic properties of its major surface glycoprotein, the HBV surface antigen (HBsAg). Subgenotypes have been identified within most of HBV genotypes. The HBV groups defined by different genotype-HBsAg subtype associations found all over the world display characteristic geographical distributions. The evolution of mutants depends on selective pressures, the replication fitness of the viral quasispecies and infected cell turnover. Sequencing of polymerase chain reaction products is recommended to identify a new HBV resistance mutation [3]. Treatment efficacy should be monitored carefully so that drug resistance, if it occurs, is detected early, before viral breakthrough and consequent disease progression resumes. Assays for serum HBV DNA and ALT should be performed 3–6 months after starting therapy to check for efficacy and compliance, the latter becoming a major contributor to primary treatment failure. Further, assays at 6-month intervals during the first 2 years of treatment are recommended for patients with milder liver disease. Three-monthly assessments are recommended after 2 years, when the probability of developing drug resistance increases. Consequences of resistance more rapidly become manifest and are more life-threatening to individuals with advanced disease, for whom regular 3-monthly assessments are recommended [3,4].

LAMIVUDINE

Lamivudine (LAM) was the first approved nucleoside analog to treat chronic hepatitis B. Drug resistance during antiviral therapy occurs with 20% rate in the first year of treatment and rises to 60-70% after 5 years of lamivudine regimen [5].

Treatment of chronic HBV infection with lamivudine often selects drug-resistant strains with single (rtM204I) or double (rtL180M+rtM204V) point mutations in the YMDD motif of HBV reverse transcriptase [6]. Other HBV polymerase mutations including rtA200V and rtV207I have been observed in patients treated with LAM [7]. A novel lamivudine-resistant strain of HBV with intact YMDD motif, which included an amino acid substitution rtA181T in the reverse transcriptase (RT) domain of HBV polymerase, has been discovered . The substitution also induced a unique amino acid substitution (HBs W172L) to overlapping hepatitis B surface (HBs) protein. The YMDD mutant strains were not detected even by using the sensitive PNA-mediated polymerase chain reaction (PCR) clamping method. The detected nucleotide substitution was accompanied by the emergence of an additional nucleotide substitution that induced amino acid change (S331C) in the spacer domain. The rtA181T mutant strain displayed a 3-fold decrease in susceptibility to lamivudine in *in vitro* experiments in comparison with the wild type. In vivo analysis using the human hepatocyte chimeric mouse confirmed resistance of this mutant strain against lamivudine [8].

Patients who develop the YMDD mutant during lamivudine therapy for hepatitis B virus (HBV) infection exhibit various clinical courses. Some patients show normal ALT levels, whereas others develop severe hepatitis exacerbations due to YMDD mutants. Negativity for HBeAg at commencement of therapy or before the emergence of YMDD mutant was an important factor among non-elevated group. More substitutions in the RT region and the other proteins may be related to the emergence of severe hepatitis caused by lamivudine-resistant virus [9].

Clinical markers appear to be very reliable indicators for the presence of LAM-R YMDD mutant HBV in CHB patients. Serum HBV DNA $\geq 1x10^6$ copies/mL by PCR assay or positive

by the hybridization assays, ongoing LAM therapy for ≥ 24 weeks, and ALT ≥ 1,2 xULN correlates strongly with the presence of LAM-R mutations in HBV isolates from the patients. LAM-R YMDD mutations were found in 98% of the baseline HBV isolates from these patients in post- liver transplantation period that presented with clinical signs of treatment failure defined above [10]. Paik et al. found out that occurrence of the therapeutic effect after 3-year course of LAM in patients HbeAg-positive. Cumulative viral breakthrough rates at 3 years were 75.0% and 14.3% in patients who had the rtM204I mutant and wild-type virus at 3 months, respectively (p=0.002) [11].

ENTECAVIR

Entecavir is a carbocyclic 2'-deoxyguanosine analog which inhibits all three HBV polymerase functions: priming, DNA synthesis and reverse transcription. Genotypic resistance to this antiviral has not been reported in lamivudine-naive patients after two-year entecavir regimen and is lower than 1% within 3 years [12-14]. Randomized, controlled study comparing entecavir 0.5 mg/day with LAM 100 mg/day in nucleoside analog-naïvepatients showed that HBeAg/HBeAb seroconversion at week 48 of the therapy was achieved by 21% vs. 18% of patients, respectively [15]. This drug is also effective in suppressing lamivudine-resistant HBV, but the activity is lower compared with wild-type HBV, even at the higher approved dose (1.0 mg). Preexisting lamivudine-resistant mutations (M204V/I+L180M) are required for the entecavir resistant phenotype to appear (about 5.8% after one-year and 10% after 2-year treatment) [16]. Three entecavir-resistant mutations have been identified: T184X, S202I/G/L and M250L/V. Each of these mutations has occurred only in the presence of preexisting lamivudine resistance mutations [17]. A phase 3 clinical study comparing 1.0 mg/day entecavir to 100 mg/day lamivudine for 48 weeks in lamivudine-refractory HBeAg-positive patients showed that at the end of the therapy, 8% of patients on entecavir lost HBeAg compared with 3% of the patients taking LAM. In addition, 19% of patients on entecavir had HBV DNA level under detection (<300 copies/mL) compared with 1% on lamivudine therapy [18].

ADEFOVIR

Adefovir dipivoxil (ADV) is an oral prodrug based on an acyclic nucleotide monophosphate analog (9-[2-(phosphonomethoxy) ethyl]-adenine, Adefovir, which acts as a DNA chain-terminator, is a selective inhibitor of viral polymerases and reverse transcriptases and has broad-spectrum antiviral activity against hepadnaviruses, retroviruses, and herpesviruses. It demonstrates antiviral activity against wild-type as well as lamivudine- and entecavir-resistant HBV mutants. Adefovir can be used as initial therapy in patients with HbeAg-positive or HBeAg-negative chronic hepatitis B, or as additional therapy in those with lamivudine-resistant HBV [19]. The cumulative percentage of patients who developed HBV genotypic resistance have been reported to be 0% in year one, 3% in year two, 11% in year three, 18% in year 4 and 28% in year 5 [20-22]. The key mutations associated with resistance to adefovir involve substitutions of the amino acid asparagine for threonine (N236T) and of alanine for valine or threonine (A181V/T). Research has demonstrated that these mutations can be associated with viral rebound, hepatitis flares, and hepatic decompensation [22-24].

Mutations within the hepatitis B virus (HBV) polymerase gene conferring drug resistance are selected during prolonged lamivudine or adefovir dipivoxil treatment. Treatments with LAM or ADV are used either sequentially or in addition, depending on treatment response or failure. Brunelle et al. investigated the possibility of the emergence of an HBV strain harboring polymerase mutations conferring resistance to both LAM (rtL180M+M204V) and ADV (rtN236T). They found that the combination of rtL180M+M204V and rtN236T mutations impairs HBV replication and confers resistance to both LAM and ADV in vitro [25].

CLINICAL IMPLICATIONS

It seems that the most important decision is the choice of the first-line therapy that we prescribe to naïve patients with HBV.

The probability that viral resistance will develop is directly proportional to the grade of selection pressure and the diversity of quasispecies. Sufficiently potent inhibition of HBV replication should be able to prevent the development and emergence of new drug-resistant variants at least in the medium term (3-5 years), mainly because mutagenesis is replication-dependent. Provided multi-site mutation is required for resistance, reducing the chance that drug-resistant virus is present prior to therapy, and if viral replication can be sufficiently suppressed by treatment, viral production will theoretically decline to a point where the creation of new quasispecies with the potential for resisting drug treatment is no longer likely. Entecavir is best used as a first-line treatment because of its potency and the high genetic barrier to resistance in patients with no prior NA treatment [26]. 94% nucleoside analog patients achieved undetectable HBV DNA level (<300 copies/mL) and <1% of such individuals experienced genotypic entecavir resistance in the third year of treatment [14]. The second potent antiviral is lamivudine. Treatment with this nucleoside analog results in a rapid decrease of serum viral load. But this improvement is transient because of the developing resistance. The emergence of this mutation limits it use in monotherapy [27].

In lamivudine-resistant patients, ADV and entecavir are both active. Adefovir alone or combined with LAM was effective. In highly viremic subjects, combined therapy was more efficacious. Baseline HBeAg positivity and/or HBV DNA 5 log were significantly associated with a lower virological response to monotherapy and with a higher rate of ADV resistance. Therefore, in patients with LAM-R adding rather than switching to adefovir should be preferred [28,29]. To maximize the efficacy of treatment in HBeAg-negative patients selecting strains resistant to lamivudine, ADV should be added to lamivudine as early as genotypic resistance is detected [30].

Entecavir is another treatment option for lamivudine-refractory patients. In such case, the dose should be higher (1mg/day) because preexisting lamivudine-resistant HBV mutations increase the risk of entecavir resistance [31].

In LAM-refractory patients exposed to entecavir, resistance to that drug and viral rebound were observed in 1% after the first year of entecavir therapy, additional 9% after the second year and additional 15% after the third year. No biochemical failure occured in these cases. In addition, 40% of lamivudine-resistant patients achieved undetectable HBV DNA level (<300 copies/mL) [14].

The most recent results suggest that treatment with combinations of two strong nucleosides with different resistance profiles may turn out to be the optimal first-line option in chronic hepatitis B [28].

The emergence of mutants that are resistant to multiple drugs is favored by the use of sequential therapies. The strategy currently recommended to prevent clinical breakthrough due to resistance is the monitoring of viral load with sensitive assays, such as quantitative PCR, in order to adjust the antiviral treatment. Close monitoring is crucial so that resistance can be detected early. For most patients, rescue therapy will be necessary and the type of treatment will depend on prior exposure to nucleoside analogs.

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