Primary resistance, multidrug resistance, and cross-resistance pathways in HBV as a consequence of treatment failure

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Abstract Antiviral resistance is now the single most important factor in treatment failure using nucleos(t)ide analogues (NA). Primary drug resistance mutations refer to amino acid change(s) that result in reduced susceptibility to an antiviral agent. Secondary compensatory mutations restore replication defects associated with primary drug resistance and may be associated with low level reduced susceptibility. Several evolutionary pathways of drug resistant HBV have been observed in patients treated with NAs. It is possible that the drug resistance mutations selected with one agent may affect the efficacy of other NAs. Several major HBV-evolutionary NA-resistance pathways (rtM204I/V, rtN236T and rtA181T/V) have now been characterised. The rtM204V/I pathway is responsible for resistance to the L-nucleosides, such as lamivudine (LMV), telbivudine (LdT) and clevudine (CLD), and also entecavir (ETV), whilst the rtN236T pathway is responsible for adefovir (ADV) and tenofovir (TFV) resistance. Both pathways are associated with clusters of secondary mutations that can affect subsequent treatment with NAs (rtT184G, rtS202I) such as ETV. The third pathway, rtA181T/V, is associated with resistance to LMV and ADV and is a potential multi-drug resistance pathway and will probably have an impact on TFV sensitivity, either alone or with the rtN236T. In naïve patients treated with ETV, at least three mutations arising at the same time are required: rtL180M + rtM204V plus either one of rtT184, rtS202 or rtM250 codon changes. Finally, in highly drug-experienced patients, clusters of mutations such as rtA181T/I233V/N236T/M250L, all on the one dominant HBV genome, are being detected which are associated with multi-drug

resistance. Sequential treatment with nucleos(t)ide analogue reverse transcriptase inhibitors (NRTI) promotes multidrug resistance. It is likely, therefore, that development of multi-drug resistance could be reduced by combination therapy optimised to individual viral phenotypes.

Introduction, background, and terminology

The problem of antiviral drug resistance is a much bigger issue in managing chronic hepatitis B (CHB) than the human immunodeficiency virus (HIV) because there is essentially only one target: the hepatitis B virus (HBV) polymerase/reverse transcriptase (rt). Similarly, only one class of specific antiviral agents has been developed for CHB: nucleos(t)ide analogues (NA).

However, within this class of drug, there are multiple groups of antiviral agents:

1. The L-nucleoside analogues: lamivudine (LMV), emtricitabine (FTC), telbivudine (LdT), and clevudine (CLD);
2. The acyclic phosphonates: adefovir (ADV) and tenofovir (TFV);
3. The cyclopentane ring group: entecavir (ETV).

This chemical classification of NA is important as it does affect the patterns and pathways of NA drug resistance.

Indications of emergence of antiviral drug resistance include:

1. Increasing viral load from nadir ($>1.0 \log_{10}$ IU/ml) in two consecutive serum samples taken 1 month apart in patients who have responded to and have been adherent to therapy with antiviral medications [1];

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- Identification of known genotypic markers of NA drug resistance within the HBV rt. Two types of mutations are observed:

- Primary resistance mutations (rtM204V/I, rtN236T)
- Secondary/compensatory mutations (rtL80V/I, rtL180M);

- Increasing serum ALT;
- Clinical deterioration.

The first indication fits the definition of virological resistance; the second indication is known as genotypic resistance; and the third and fourth points include clinical resistance.

The annual prevalence of resistance rates for lamivudine, adefovir, entecavir, emtricitabine, and telbivudine is shown in Table 1.

Pathways and patterns of resistance

The primary resistance mutations associated with drug failure for HBV are shown in Fig. 1. As can be seen, eight codons in the HBV polymerase account for primary treatment failure in CHB. Prospective analysis of these eight primary resistance mutations has revealed that they can be sorted on the basis of the group of NA (e.g., lamivudine, acyclic phosphonate, or cyclopentane sugar group) into particular pathways (Fig. 2).

To date, five pathways have been identified:

- The L-nucleoside pathway (rtM204V/I), whereby lamivudine, emtricitabine, telbivudine, and clevudine

Fig. 1 Primary resistance mutations for lamivudine (LMV), telbivudine (LdT), adefovir (ADV), tenofovir (TDF), and entecavir (ETV) mapped onto the HBV polymerase gene

Primary Resistance Mutations: The Important Codons

B-domain	169					C-domain	202	
	180						204	
	181							
	184							
D-domain	236					E-domain	250	
TOTAL					8			
	169	180	181	184	202	204	236	250

Proposed Key Pathways:

• rtM204V/I	→	L-NUCLEOSIDES (LMV, LdT)
• rtN236T	→	ETV in LMV-Experienced
• rtA181T/V	→	ACYCLIC PHOSPHONATES (ADV, TDF)
• rtL180M+rtM204V+T184 or S202 or M250	→	ETV (naïve)
• rtA181T+rtL233V+rtN236T+rtM250L	→	MDR

Fig. 2 The proposed pathways for the L-nucleosides (LMV, LdT), acyclic phosphonates, and shared and multidrug resistance (MDR) linked to the eight codons associated with nucleos(t)ide analogues (NA) drug failure

Table 1 Annual prevalent resistance rates for lamivudine, adefovir, entecavir, emtricitabine, and telbivudine

Drug	Resistance at year of therapy expressed as percentage of patients				
	1	2	3	4	5
Lamivudine ^a	23	46	55	71	80
Adefovir ^b (naïve HBeAg-neg)	0	3	11	18	29
Adefovir (LAM resistant) ^c	0–18	–	–	–	–
Entecavir ^d (naïve)	0.1	0.4	1.1	1.1	–
Entecavir ^d (LAM resistant)	6	15	35	43	–
Emtricitabine ^d	9–16	19–37	–	–	–
Telbivudine ^e (HBeAg-pos) ^f HBeAg-neg ^f	4.4 ^d	2.16 ^e			
	2.7	8.6			

^a Modified and updated from Lai et al. [2] and Leung et al. [3]

^b From Locarnini and Mason [4]; Hadziyannis et al. [5]

^c From Lee et al. [6]

^d From Perrillo [7] and Colonno et al. [8]

^e In the LAM comparator arm, the percentage was only 8% based on a complex case definition of antiviral drug resistance/treatment failure. One would thus expect a comparable relative level (Lok and McMahon [9])

^f Of 10–12% based on genotypic resistance compared with lamivudine (25% per annum)

- treatment select out the rtM204V/I. This pathway includes entecavir in lamivudine-experienced patients.
- The acyclic phosphonate pathway (rtN236T), in which adefovir and tenofovir treatment select out and/or consolidate the rtN236T HBV quasiespecies [10, 11].
 - A shared pathway (rtA181T/V), whereby treatment with either L-nucleosides or acyclic phosphonates can result in selection of HBV quasiespecies with rtA181T/V. This pathway is seen in about 40% of adefovir failure and less than 5% of lamivudine failure.
 - Naïve entecavir resistance pathway (rtL180M + rtM204V with one of either rtT184, S202, or M250 codon changes). In this pathway, three mutations are required to appear simultaneously accounting for the very low resistance profile of entecavir [12].
 - Multidrug resistance pathways. Complex patterns and clusters of specific mutations in HBV polymerase associated with multidrug failure. A recent example includes rtA181T + rtI233V + rtN236T + rtM250L. It is important to note that the rtI233V and M250L substitutions in isolation do not confer significant drug resistance nor significantly reduce replication capacity in the absence of selection pressure, but appear to act to compensate for the replication defects associated with acquisition of multidrug resistance.

Actual composite virological examples from groups of patients failing lamivudine, telbivudine, adefovir, or entecavir are shown in Fig. 3. As can be seen, a number of

compensatory mutations are accumulated in patients undergoing antiviral therapy in the face of primary drug resistance. A number of these compensatory or secondary resistance mutations can result in cross-resistance with other groups of NAs even when the patient has not been treated with that particular agent. For example, rtT184/S202/M250 changes, which appear on lamivudine, affect sensitivity to entecavir. Two examples of multidrug-resistant HBV isolates are shown in Fig. 4.

Polymerase-HBsAg overlap

Because of the circular nature and open-reading frame (ORF) arrangement of the genes of HBV, the viral envelope (HBsAg) gene overlaps completely within the reverse transcriptase gene. NA resistance can result in changes in HBsAg (Fig. 5). This polymerase-envelope overlap is important as Torresi and colleagues [13] have shown that the common lamivudine-resistant HBVs (rtV173L + rtL180M + rtM204V) have important and significant changes in HBsAg (sE164D + sI195M) which significantly reduce anti-HBs (vaccine-associated) binding in vitro (see Table 2). Likewise, in adefovir failure, the rtA181T HBV can be found either by itself or in association with rtN236T, in up to 20% of cases, but the rtA181T results in an sW172[STOP] in HBsAg. Warner and Locarnini have shown that this HBV quasiespecies is defective

Fig. 3 Pathways of HBV quasiespecies evolution observed in patients failing (a) L-nucleosides such as lamivudine (LMV) or (b) telbivudine (LdT), (c) the acyclic phosphonates such as adefovir (ADV), and (d) the cyclopentane ring group such as entecavir (ETV). X, cross-resistance; P, partial response; S, sensitive

Fig. 4 Two recently characterized pathways of multidrug resistance in HBV. These pathways emerge when patients are placed on sequential monotherapies. X, cross-resistance; ?, unknown sensitivity

in virion secretion [14], is retained in the cell, and acts as a dominant negative mutant for wild-type HBV secretion [14]. The clinical implication of these observations is that the virological case definition of drug resistance, >1.0 log IU/ml from nadir in two consecutive samples taken 1 month apart [1], does not hold if this mutant is (co)-selected out. The viral load, following first appearance of rtA181T, increases only very gradually from nadir over 12 months. The practical implication of this finding will be the need for HBV genotyping and polymerase sequencing, as well as HBV viral load, in patients undergoing antiviral

Table 2 Polymerase and envelope link

Envelope mutants	Polymerase mutant ^a	Ag–Ab binding [IC ₅₀ (g/ml)]
Wild type	Wild type	1.09
<i>HBIG-Escape</i>		
sG145R	rtW153Q (W499Q)	>55.0
sD144E/G145R	rtG153E (G499E)	>55.0
<i>Drug resistant</i>		
sF158Y	rtF166L	1.86
sE164D	rtV173L	14.86
sW196S	rtM204I	8.29
sI195M	rtM204V	5.26
sM198I	rtV207I	12.5
sE164D/I195M	rtV173L/rtL180M/rtM204V	54.53

Note: The antibody binding data are modified from Torresi et al. [13]

^a rtL180M (L526M) in polymerase causes NO change in envelope

therapy. This is especially relevant in patients co-infected with HIV where lamivudine is widely used and also in those patients treated with ADV (CHB mono-infected) or TDF.

Summary and future directions

The current emerging patterns of antiviral drug resistance to the HBV polymerase are complex. However, the eight codons associated with primary drug resistance fall into five major pathways, which appear reasonably predictable based on the analysis so far. However, broad clusters of compensatory mutations, especially during lamivudine therapy, are compromising future salvage therapy options.

Fig. 5 Polymerase-envelope overlap in the HBV genome highlighting that drug-resistant changes in polymerase can cause potentially important changes in HBsAg with all the NA currently available

Algorithms for the use of viral load and HBV genotyping/polymerase sequencing are clearly needed to be developed for patients receiving antiviral therapy. There is also a strong need for the availability and more widespread use of interactive database programs to guide rescue therapy in CHB.

Finally, the unique genetic arrangement of the HBV genome with the polymerase and surface genes overlapping has substantial public health and diagnostic implications and relevance. Altered HBsAg due to the emergence of antiviral drug resistance may also have pathological consequence.

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Review—suggested reading

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