Viral Resistance in HBV

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Introduction

The hepatitis B virus (HBV) has evolved a unique life cycle that results in the production of enormous viral loads during active replication without actually killing the infected cell directly. The virus copies its genome by the process of reverse transcription and, as a consequence, mutant viral genomes are generated continuously. Particular selection pressures, both endogenous (host immune clearance) and exogenous (vaccines and antivirals), readily select out so called "escape" mutants that have a replicative advantage in the presence of the selection pressure. Antiviral drug escape or drug resistance reflects reduced susceptibility of a virus to the inhibitory effect of that drug and typically results from a process of adaptive mutations under therapy. An important concept in understanding this escape phenomenon is that single or double mutations associated with resistance generally pre-exist, whilst three or four mutation profiles require ongoing selection and thereby replication in the presence of the particular drug selection pressure. Since most nucleoside/nucleotide analogues fail therapeutically when one (rtN236T) or two mutations (rtL180M+rtM204V) emerge as the dominant viral population, the chance that three or four mutations pre-exist is much lower and makes a strong argument for the use of combination chemotherapy for hepatitis B. This is known as the combinatorial ledge (Colgrove and Japour (1999) *AVR*;41:45).

Causes of HBV Drug Resistance

In hepatitis B, antiviral drug resistance depends on at least five factors: (1) viral mutation frequency, (2) magnitude and rate of virus replication, (3) selective pressure exerted by the drug, (4) replication fitness of the mutant, and (5) availability of replication space (Locarnini, S. 2005. *Seminars In Liver Disease*; 25(Suppl.1):9). The development of drug resistance is not unexpected if viral replication continues in the setting of ongoing treatment, especially monotherapy. Indications of emergence of drug-resistant HBV include: (i) increasing viral load (≥ 1.0 log IU/ml) and, (ii) identification of known genotypic markers of drug-resistance within the viral polymerase (Locarnini, S. et al. 2004. *Antivir Ther*;9:679-693).

Nucleos(t)ide Analogues and Patterns of Resistance

Two types of mutations in the HBV polymerase can be identified:

(i) discriminatory mutations (LMV: rtM204V/I), and
(ii) (ii) compensatory mutations (LMV:rtV173L).

Furthermore, three chemical groups within the class of nucleos(t)ide analogue can be recognised:

(i) L-Nucleoside Group (lamivudine, emtricitabine, telbivudine, clevudine);
(ii) Acyclic Phosphonate Group (adefovir, tenofovir);
(iii) Cyclopentene/Cyclopentane Group (entecavir/abacavir).

This chemical classification has relevance to the drug-resistant patterns observed during treatment failure, both in terms of discriminatory versus compensatory mutations. For the L-nucleosides, the major discriminatory mutation directly associated

with resistance is the rtM204V/I in the YMDD motif of the C-domain of the HBV polymerase. Once selected, this mutation effectively "burns" all other L-nucleosides. Another discriminatory mutation associated with Lamivudine failure is rtA181T/V (Yeh et al. 2000. *Hepatology*;31:1318) which is also cross-resistant with Adefovir. Long-term use of Lamivudine monotherapy is associated with the selection of a number of compensatory mutations, including rtI169T, rtV173L (Delaney et al. 2003. *JV*;77:1833) rtT184S, and rtV214A/rtQ215S (Bartholomeusz et al. 2005. *Hepatology*; 42(Suppl.1)594A), which directly increase the IC_{50} for Entecavir, Adefovir, and Tenofovir, compromising the use of these agents subsequently as "rescue" therapy for patients with lamivudine-resistant hepatitis B.

Conclusions

Prevention of resistance requires the adoption of strategies that effectively control virus replication. For example, the use of antiviral drugs that require the selection of at least three or four mutations or more in the viral polymerase to occur in order to result in treatment failure, (i.e., increasing the genetic barriers for resistance). Also, the use of combinations of drugs that act together at least additively or synergistically against HBV would certainly reduce the rate of emergence of drug resistance and provide an environment of longer term therapeutic control of viral replication (Zoulim F. 2004. *Antiviral Res*;64:1-15). However, if the practice of maintenance Lamivudine monotherapy in the setting of resistance continues, then the diversity of HBV quasispecies found in the patient will continue to expand and include dominant isolates cross-resistant to all nucleos(t)ide analogues in the hepatitis B armamentarium. HBV polymerase sequencing will be required to determine the various patterns of quasispecies that have been generated in order to guide the choice of rescue therapy, if there is one.