MECHANISMS OF HEPATITIS B VIRUS DRUG RESISTANCE

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With the development of new antiviral agents, drug resistance is one of the most challenging issue in the management of chronic hepatitis B therapy. The main antivirals available for the treatment of chronic hepatitis B belong to the nucleoside analog family. They may select for viral polymerase gene mutants that exhibit a lower susceptibility to the drug. This usually results from a lower binding activity of the compound in the catalytic site of the enzyme or from excision of the drug, among the potential mechanisms.

There are several viral and host determinants involved in the selection of HBV drug resistant mutants.

Viral dynamics are rapid for HBV with a daily production of virions in the range of 10^{12}-10^{13} for HBV. In the recent reports, the half-life of free HBV particles varies between 3 and 24 hours. The half-life of HBV infected hepatocytes has been estimated to be up to 100 days, with large lifespan heterogeneity, which is one of the major reasons for the requirement of long-term therapy in chronic hepatitis B.

Mutations occur spontaneously during the replication of HBV. The HBV reverse transcriptase is intrinsically error prone and lack proofreading function, allowing for frequent replication errors to occur. The result is the generation of multiple viral variants, known as a quasi-species, that coexist and reach population densities in direct proportion to their relative replication fitnesses. It has been predicted that every nucleoside of the 3.2 Kb HBV genome theoretically can be substituted every day within a given infected patient.

Since any drug pressure may act to select pre-existing drug resistance viral variants, the speed for selecting drug resistance mainly depends on the turnover of the viral nucleic acid acting as source of new viral genomes. Viral genome mutations are supposed to be archived in cccDNA present within the nucleus of infected hepatocytes as extra-chromosomal (episomal) material. HBV cccDNA is relatively stable within infected hepatocytes. The time needed for selecting drug resistance mutations, present at baseline only as minority genomic variants, to expand and fill a major part of the virus population is relatively long for HBV. It depends on the fitness of the mutant viruses in the presence of the drug and the replication space available in the liver for the spread of the mutants. In chronic hepatitis B, the replication space is provided by hepatocyte turnover, which allows the loss of HBV wild-type infected cells and the generation of non-infected hepatocytes that are susceptible to new HBV mutant infections. This process is usually very slow in chronic hepatitis B because the immune mediated killing of infected cells is slow.

In a situation in which most potential target cells are already infected and releasing virions, it is clear that infected cells with a long half-life will provide only a minimal opportunity for replacing the original virus population by a new one of drug-resistant variants. This is the case for HBV, whose infected hepatocytes may survive for several weeks or months. This is one of the reasons why the dynamics of selection of drug resistance are so different comparing HBV and HIV.

Viral fitness is another important factor in the mechanism of selection of drug resistant mutants. This includes replication advantage conferred by the mutations in the presence of the drug, but also the adverse effect of the mutations on the replication capacity of the mutants. The polymerase gene mutations may confer a decreased susceptibility of HBV mutants to the drug; resistance may need a sequential addition of mutations to achieve higher levels of resistance, as this may be the case for entecavir (primary resistance mutations at position rt204, and secondary resistance mutations at position rt184, rt202 or rt250). Many of the main resistance mutations are associated with compensatory resistance mutations to restore the replication capacity of the mutant virus. The newer generation of antivirals seems to have a higher genetic barrier to resistance (i.e., number of mutations needed for the establishment of resistance and/or resistance mutations resulting in reduced viral fitness). The kowledge of the cross-resistance profile of the antiviral drugs is also very important to choose antivirals for combination of add-on therapy.

Apart from the direct effect of the polymerase gene mutations on the enzymatic activity of the viral polymerase, which may result in impaired replication capacity, there are conservatory constraints due to the fact that the HBV genome shows overlapping reading frames. Changes at one position in the viral polymerase may affect the structure and function of the surface protein. Therefore it was shown that some lamivudine-associated resistance mutations may modify the antigenicity of the HBV surface antigen. HBV escape mutants induced by antiviral therapy have recently attracted much attention because of their potential spread in the population. Moreover, mutants of the viral polymerase gene may induce mutations in the overlapping surface antigen which may then generate defective or less infectious mutants, that may need trans-complementation of the mutant protein by wild type to package and propagate the mutant virus (some of the M204I and the A181T mutants are examples). The impact of the

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specific antiviral immune response on the selection of drug resistant mutants is not well known.

There are major clinical implications from these fidings. Antiviral therapy should be based on nucleoside analogs exhibiting a potent antiviral effect and having a high genetic barrier to resistance to limit the emergence of drug resistance. In second line treatments, add-on strategies using on drugs with a complementary cross-resistance profile are preferred to inhibit the replication of the major variants of the viral quasi-species. The knowledge of the kinetics of the resistance mutant emergence has also implication in the virologic monitoring of antiviral treatment. All these informations should guide clinicians in the management of chronic hepatitis B therapy.

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