ANTIVIRAL RESISTANCE IN HBV

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In the past 10 years, significant progress has been achieved in the management of chronic hepatitis B with the successive development of six potent antiviral medications (lamivudine, adefovir dipivoxil, pegylated interferon alpha, entecavir, telbivudine and tenofovir). However, the clinical results of antiviral therapy have been limited by the emergence of antiviral drug resistance especially with the first generation of nucleoside analogs (lamivudine, adefovir and telbivudine)[5]. Furthermore, the unique mechanism of viral genome replication and persistence within infected cells is responsible for viral persistence even after prolonged therapy with the newer antivirals (entecavir and tenofovir). This is the major reason why life-long treatment is envisaged in the majority of patients, which may expose them to long-term risk of developing resistance.

HBV persistence in infected hepatocytes is due to the long half-life of hepatocytes, the defect in the specific anti-HBV immune response, the persistence of cccDNA in the nucleus of infected cells and viral genome variability[7, 17]. Molecular studies of HBV have shown that simple mutants pre-exist in the overall population of hepatitis B viruses prior to therapy. This intrinsic genetic variability is the consequence of spontaneous errors occurring during the reverse transcriptase step at each replication cycle. This is due to the fact that the hepatitis B virus does not possess DNA repair mechanisms that maintain the stability of viral DNA during replication by the elimination of incorrectly inserted nucleotides. Thus, considering the high levels of virus production that characterizes HBV infection, this results in an important heterogeneity of the viral genome, and the generation of numerous viral mutants which circulate in the patients' blood and which are called quasispecies. Viral quasispecies within the same patient evolve during the course of infection, different variants or mutants being selected at different stages of infection in response to the host immune response or antiviral therapy, depending on their intrinsic fitness. During antiviral therapy, a complex mixture of genetically distinct variants may develop under selective pressure. A newly acquired or a pre-existing mutation conferring a selective advantage to a variant will generate a virus, which is more viable and can spread more rapidly in the liver, allowing the corresponding mutant to accumulate and become the dominant species in the infected liver in the presence of the antiviral drug. The kinetics of replacement of wild-type virus in liver cells by a dominant mutant are generally slow[6]. Indeed, resistant mutants mainly infect uninfected cells. The spread of the dominant mutant therefore depends on the availability of a free liver space for its replication. Therefore, several months of antiviral therapy may be needed for the immune system to remove the hepatocytes containing wild-type virus and to generate new cells that are susceptible to infection by viral drug-resistant mutants[8, 9, 16]. On the other hand, the infectivity of antiviral drug-resistant mutants may have a major impact on the rapidity of selection of these strains during therapy. For instance, some mutations in the overlapping surface gene may result in reduced viral export and infectivity[15]. The level of resistance to a drug conferred by a given mutation may have profound implication on the fitness of the mutant. This may explain the difference in drug resistance rates observed with the different antivirals.

The use of *in vitro* phenotypic assays has been crucial for the characterization of newly identified resistant mutants and determine their cross-resistance profile[4]. Results allowed to understand the different mechanism of viral resistance to lamivudine and adefovir[1, 11], the mechanism of primary failure to adefovir therapy[3], the unique mechanism of entecavir resistance[2, 10, 13], and to characterize the emergence of multi-drug-resistant strains in patients receiving sequential antiviral therapy[14]. The cross-resistance profile for the main resistant mutants was determined which allowed to provide recommendation to clinicians for treatment adaptation based on molecular virology data[5, 18].

The role of infectivity and viral fitness in the process of selection of drug-resistant mutants was studied in HBV susceptible HepaRG cells. It was shown that mutations in the viral polymerase gene conferring drug resistance may also induce mutations in the overlapping envelope gene and in turn may alter viral infectivity[12]. These results may be highly relevant for the development of clinical strategies aiming at preventing drug resistance or at selecting variants with impaired fitness.

The understanding of the development of HBV drug resistance has allowed to significantly improve the management of antiviral resistance and to design better treatment strategies to prevent resistance. The current standard of care relies on treatment initiation with antivirals combining a strong antiviral potency and a high barrier to resistance. A precise virologic monitoring is required to measure antiviral efficacy, and to diagnose partial response or viral breathrough at an early stage. This allows to adapt antiviral treatment preferrably using an add-on strategy with

Recebido em 29/05/2009 Aceito em 09/06/2009
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Gazeta Médica da Bahia 2009;79 (Supl.2):43-44
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a drug having a complementary cross-resistance profile. This strategy has been shown to be efficient in controling viral replication and preventing liver disease progression in the majority of patients[5, 18].

The future challenge will be to determine whether de novo combination of nucleoside analogs belonging to the new generation of drugs will provide an added benefit in terms of drug resistance and prolonged viral suppression. The identification of new antiviral targets will be important in that respect to develop more potent combination strategies.

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