Genotypic resistance to lamivudine among hepatitis B virus isolates in Mexico

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Background: Drug resistance of hepatitis B virus (HBV) is an increasing clinical problem. Resistance to lamivudine in HBV isolates in Mexico has been poorly explored.

Objectives: To characterize the mutation patterns associated with genotypic resistance to lamivudine and their prevalence among HBV isolates in Mexico.

Material and methods: Thirty-nine Mexican HBV isolates were analysed by PCR and line probe assay for detection of genetic variants in the polymerase open reading frame domains B and C (INNO-LiPA HBV DR; INNOGENETICS N. V., Ghent, Belgium). This assay detects wild-type and mutations at codons 180, 204 and 207 of the HBV polymerase gene, and at codon positions 171, 172, 195, 196, 198 and 199 of the HBV surface antigen (HBsAg). HBV isolates were obtained from HBsAg-positive serum samples of 15 chronic hepatitis patients, two haemodialysis patients with chronic HBV carriage, 20 men found positive for HBsAg when seeking HIV testing and two AIDS patients with chronic HBV infection. None of the participants had received antiviral therapy.

Results: Overall, HBV wild-type was found in 37 (94.9%) out of the 39 isolates studied. Two (5.1%) out of the 39 isolates showed mixed wild-type and mutant populations. These mutations occurred in isolates from one hepatitis patient and one haemodialysis patient. The isolate from the hepatitis patient showed a double mutation at codon positions 180 (L180M) and 204 (M204V), thus a 2.6% prevalence of genotypic resistance to lamivudine was found. The isolate from the haemodialysis patient showed a single mutation at codon position 180 (L180M). The two HBV mutant isolates were further analysed for genotype and both isolates were genotype H.

Conclusions: HBV genotypic resistance to lamivudine exists in Mexican isolates. The results highlight the importance of testing for HBV resistance before treatment and have implications for a more rational use of drugs.

Keywords: drug resistance, mutations, viral infections

Introduction

Hepatitis B virus (HBV) is a double-stranded DNA virus of the Hepadnaviridae family.1 HBV is an important pathogen responsible for morbidity and mortality worldwide. Reports indicate that about two billion people are infected by HBV in the world, and 350 million of them suffer from chronic infections.2,3 Morbidity and mortality in chronic HBV infection is linked to development of liver cirrhosis and hepatocellular carcinoma.2,4 Antiviral treatment is the only way to reduce morbidity and mortality from chronic HBV infection.2 The aim of antiviral treatment is to control HBV replication by keeping a durable suppression of serum HBV DNA to the lowest level possible and to cure liver disease by avoiding the progression of chronic hepatitis to cirrhosis and the end-stage complications of cirrhosis.2,5 Current antiviral drugs for the treatment of chronic hepatitis B include interferon, lamivudine and adefovir dipivoxil.2,6–8 Interferon treatment leads to a more durable response but is associated with unpleasant side effects.8 When interferon is contraindicated or ineffective, lamivudine or adefovir dipivoxil are given.5 However, lamivudine or adefovir dipivoxil are

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also approved as initial therapy for chronic hepatitis B. Lamivudine, famciclovir and adefovir dipivoxil are nucleoside analogues that mainly act by inhibition of HBV polymerase activity resulting in a decrease in viral replication. Lamivudine is effective and well tolerated but requires long-term therapy and is associated with drug resistance. Famciclovir can be used in selected patients with lamivudine resistance, and may be beneficial in patients with hepatitis B post-orthotopic liver transplantation. Adefovir dipivoxil has proven efficacy and a very low rate of drug resistance but is associated with a small risk of reversible nephrotoxicity. A combination therapy may prove to be more effective than monotherapy in suppressing viral replication and may decrease or delay the incidence of drug resistance.

Lamivudine and famciclovir resistance of HBV has been associated with mutations of the polymerase gene. According to the new nomenclature for antiviral-resistant human HBV mutations in the polymerase region, lamivudine- and famciclovir-related resistance mutations are located in at least codon positions 180, 204 and 207. Lamivudine therapy is not currently used in many developing countries, and little is known about the primary resistance to this drug worldwide. In Mexico, drug resistance of HBV has been poorly explored. Therefore, we sought to determine the prevalence of genotypic resistance to lamivudine in HBV isolates obtained from untreated HBV-infected individuals in Mexico.

Materials and methods

HBV isolates

Thirty-nine Mexican HBV isolates, obtained from serum samples of HBV surface antigen (HBsAg)-positive subjects without evidence of lamivudine or famciclovir treatment, were analysed. Of the 39 HBV isolates, 15 were obtained from patients suffering from chronic hepatitis, two from haemodialysis patients with chronic HBsAg carriage, 20 from asymptomatic men found positive for HBsAg when seeking HIV testing and two from AIDS patients with chronic HBV infection.

HBV DNA amplification and drug resistance detection

HBV DNA was extracted from 200 µL of serum by using the High Pure PCR Template Preparation Kit (Roche Applied Science, Penzberg, Germany) as recommended by the manufacturer. HBV DNA was amplified with pre-S primers by nested PCR under the following conditions: 40 cycles at 94°C for 30 s, 45°C for 30 s and 72°C for 30 s. Determination of HBV drug resistance was performed by INNO-LiPA HBV DR (INNOGENETICS N. V., Ghent, Belgium) following the instructions of the manufacturer. This assay is based on the reverse hybridization principle and detects wild-type and mutations or polymorphisms at codons 180, 204 and 207 of the HBV polymerase gene. In addition, changes at codon positions 171, 172, 195, 196, 198 and 199 of the HBsAg can be detected due to the overlapping reading frame.

Results

HBV drug resistance

All 39 HBV isolates were successfully analysed by INNO-LiPA HBV DR. Overall, HBV wild-type was found in 37 (94.9%) out of the 39 isolates studied, while mixed HBV wild-type and mutant populations were found in two (5.1%) isolates. Mutations were observed in one chronic hepatitis patient and one haemodialysis patient with chronic HBV infection. The isolate from the hepatitis patient showed a double mutation at codon positions 180 (L180M) and 204 (M204V), and therefore, was resistant to lamivudine and famciclovir, while the isolate from the haemodialysis patient showed a single mutation at codon position 180 (L180M).

Discussion

We found that HBV with a double mutation at codon positions 180 (L180M) and 204 (M204V), resistant to lamivudine, circulated in 2.6% of the untreated HBV-infected subjects studied. An additional 2.6% of HBV with a single mutation at codon position 180 (L180M) that predisposes to a more rapid evolution towards full lamivudine resistance was found. These prevalences were unexpected since lamivudine is not currently used for treatment of HBV infection in Mexico. In principle, spontaneous HBV mutations conferring drug resistance are rare, and infection by a drug-resistant HBV strain may rather occur through transmission from a lamivudine-treated HBV-infected patient. Some possible explanations for the presence of resistance to lamivudine in the naïve untreated Mexican HBV-infected subjects could be provided. In a country where lamivudine is not currently in use for hepatitis B, we may speculate that emergence of drug resistance came from an infection with an imported drug-resistant HBV strain. Besides, resistance might occur when HBV-infected subjects are treated with lamivudine for other viral infections during a current—and some times unapparent or unknown—HBV infection. The latter can be supported by the fact that lamivudine may be used also for treatment of HIV. However, although it is possible, it was not probable in the drug resistance case since the patient carrying the lamivudine-resistant HBV strain was not coinfected with HIV.

Reports on prevalence of HBV primary resistance to lamivudine and famciclovir are scarce. A study performed with randomly selected chronic carriers from Spain revealed a 3.8% prevalence of lamivudine and famciclovir resistance. Similarly, primary infection with a lamivudine-resistant HBV has been reported in France. In Japan, a study performed with 18 chronic HBV carriers who had not had any experience with antiviral agents revealed that five of them showed mutations associated with lamivudine resistance. Thus, our frequency of HBV genotypic resistance to lamivudine and famciclovir among individuals not currently treated and without any evidence of lamivudine treatment in the past is lower than that found in Japan, and comparable to that found in Spain.

We performed genotyping of both HBV isolates with mutations by INNO-LiPA HBV Genotyping (INNOGENETICS N. V.). Interestingly, both isolates were genotype H. This genotype is found at high frequency in Mexico and Central American countries. Mutations associated with lamivudine resistance have been found in HBV genotypes A, B, C and D. To the best of our knowledge, this is the first report on resistance to lamivudine in HBV genotype H.

We have used the line probe assay because it reliably detects both lamivudine and famciclovir resistance mutations in HBV strains. This method has shown highly comparable results with sequencing, but the line probe assay may better detect wild-type and mutant viruses mixed populations than sequencing. The line probe assay has shown high sensitivity in detecting HBV DNA down to a concentration of 10^3 copies/mL.
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addition, the probes used in the line probe assay have been shown to be very specific, and may detect a mixed wild-type and mutant virus population earlier than sequencing.

We conclude that HBV genotypic resistance to lamivudine in untreated HBV-infected subjects in Mexico exists. Lamivudine-resistant isolates were HBV genotype H. Lamivudine resistance testing before treatment is a rational practice for optimal planning of therapeutic schemes.

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Transparency declarations

None to declare.

References