World Journal of Gastroenterology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.3748/wjg.v20.i45.17100

World J Gastroenterol 2014 December 7; 20(45): 17100-17106
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

RESEARCH REPORT

Resistant mutants induced by adefovir dipivoxil in hepatitis B virus isolates

Su-Wen Jiang, Li-Peng Yao, Ai-Rong Hu, Yao-Ren Hu, Shi-Xiang Chen, Tao Xiong, Guo-Sheng Gao, Xiao-Yue Liang, Shi-Xiong Ding, Peng-Jian Weng

Su-Wen Jiang, Ai-Rong Hu, Yao-Ren Hu, Tao Xiong, Guo-Sheng Gao, Xiao-Yue Liang, Shi-Xiong Ding, Peng-Jian Weng, Institute of Liver Diseases, Ningbo No. 2 Hospital, Ningbo 315010, Zhejiang Province, China
Li-Peng Yao, Ai-Rong Hu, Department of Molecular Biology, School of Medicine, Ningbo University, Ningbo 315010, Zhejiang Province, China
Shi-Xiang Chen, Department of Infectious Diseases, No. 113 Hospital of the PLA, Ningbo 315010, Zhejiang Province, China
Author contributions: Jiang SW, Hu AR and Gao GS participated in research design; Gao GS and Ding SX tested the specimens; Weng PJ tested the specimens and collected the data; Jiang SW, Yao LP, Hu YR, Chen SX, Xiong T and Liang XY collected and analyzed the data; Gao GS analyzed the data and drafted the manuscript; Jiang SW and Hu AR drafted the manuscript.
Supported by BaoenWang Liver Fibrosis Research Fund of the Chinese Foundation for Hepatitis Prevention and Control, No. 20100009; Zhejiang Medicine and Health Sciences Research Fund, No. 2008B167 and No. 2014PYA018; Nanjing Military Area Command of the PLA Medical Technology Innovation, No. 11MA019; Ningbo Health Technology Project for Excellent Young and Middle-aged Talent, No. 2011-145; Ningbo Leader and Top Notch Person Training Project, No. 2012-131; and Zhejiang Medicine and Health Project for Excellent Young Talent, No. 2013-245
Correspondence to: Ai-Rong Hu, MD, Institute of Liver Diseases, Ningbo No. 2 Hospital, 41 Xibei Street, Haishu District, Ningbo 315010, Zhejiang Province, China. huairong6666@126.com
Telephone: +86-574-83870605 Fax: +86-574-83870626
Received: May 28, 2014 Revised: July 17, 2014
Accepted: August 13, 2014
Published online: December 7, 2014

Abstract

AIM: To investigate the loci of adefovir dipivoxil (ADV)-induced resistance in hepatitis B virus (HBV) isolates and optimize the management of ADV-treated patients.

METHODS: Between June 2008 and August 2010, a cross-sectional control study was conducted comprising 79 patients with chronic HBV infection-related liver disease who had been administered ADV monotherapy. Patients underwent liver imaging. Serum DNA extracts were analyzed for HBV DNA levels, genotypes, and serology markers, and deep sequencing of the HBV P gene was performed.

RESULTS: ADV-resistant patients were found either with a single mutated locus, or with coexisting mutated loci. The most prevalent mutations were rtA181T, rtV214A, and rtN236T. Twenty-six patients had more than two mutated loci. The mutants were distributed among the patients without any significant affinity for gender, age, end-stage of liver disease, complications of non-alcoholic fatty liver disease, or HBV DNA levels. Patients with the rtA181T mutant were primarily infected with genotype C and e-antigen negative HBV, while patients with the rtN236T mutant were primarily infected by genotype B HBV (χ^2 = 6.004, 7.159; P = 0.023, 0.007). The duration of treatment with ADV was shorter in the single mutant group compared with the multi-mutant group (t = 2.426, P = 0.018).

CONCLUSION: Drug-resistant HBV mutants are complex and diverse. Patients should receive the standard and first-line antiviral treatment, strictly comply with medication dosage, and avoid short-term withdrawal.

Key words: Hepatitis B virus; Adefovir dipivoxil; Drug-resistant mutant; Gene sequencing

Core tip: Currently, hepatitis B virus (HBV) resistance to adefovir dipivoxil (ADV) has become more prevalent. However, it is not known whether drug-resistant

HBV mutants induced by ADV are related to the clinical features of patients. In this study, we analyzed the exhaustive resistant mutants induced by ADV. Of 79 patients, 26 (32.9%) had more than two loci of drug resistance in HBV mutants. Because drug-resistant HBV mutants induced by ADV are complicated and diverse, we conclude that testing for resistant mutants prior to antiviral treatment, administering standard and first-line antiviral treatment, and personalizing medication according to the clinical characteristics of patients is important to avoid subsequent noncompliance and the need for salvage treatment.

Jiang SW, Yao LP, Hu AR, Hu YR, Chen SX, Xiong T, Gao GS, Liang XY, Ding SX, Weng PJ. Resistant mutants induced by adefovir dipivoxil in hepatitis B virus isolates. *World J Gastroenterol* 2014; 20(45): 17100-17106 Available from: URL: http://www.wjgnet.com/1007-9327/full/v20/i45/17100.htm DOI: http://dx.doi.org/10.3748/wjg.v20.i45.17100

INTRODUCTION

Patients with chronic hepatitis B virus (HBV)-related liver disease should be treated in a timely manner to prevent progressive HBV recurrence[1-5]. However, treatment is limited by the ever-increasing number of drug-resistant HBV mutants resulting from the wide use of nucleoside/nucleotide analogues (NUCs)[6-11]. Adefovir dipivoxil (ADV) is an NUC that is relatively effective and often used in salvage therapy for HBV-related liver disease. Yet the viruses' resistance rate continues to rise[12] and its five-year cumulative resistance rate is 29%[1,2].

China is an endemic area for HBV infection. In China, ADV is routinely applied in initial monotherapy for patients with HBV-related liver disease, as well as monotherapy to patients with NUC-resistant HBV. As a result, HBV resistance to ADV has become more prevalent[13], and research on ADV drug resistance has attracted extensive attention. Currently, the HBV mutations rtA181V/T/S and rtN236T are considered the primary sites of ADV-associated drug resistance[1,2,5], but others continue to be found[14-16].

HBV comprises eight genotypes (A to H). Their differentiation is based on a divergence in the entire nucleotide sequence that is greater than 8%[17]. Approximately 95% of Chinese HBV patients carry the most common genotypes, B and C. There is no consensus on the relatedness of HBV genotype and ADV resistance.

Most current studies focus on ADV drug resistance and optimal treatment. However, there has been no report concerning the association between the clinical features of patients and drug-resistant HBV mutants induced by ADV. This study analyzed the loci of mutations in HBV strains in which resistance had been induced by ADV treatment, in patients with chronic HBV infection-related liver diseases. The study provides a valuable reference for standard and optimal treatment of NUCs.

MATERIALS AND METHODS

Study design and patients

This was a cross-sectional control study of ADV-resistant HBV mutants induced by ADV monotherapy. The study included 281 ADV monotherapy patients treated at Ningbo No. 2 Hospital between June 2008 and August 2010, 79 of these patients had ADV-resistant HBV mutants. Patients included 66 men and 13 women, 20 to 68 years old, with a median age of 43.2 ± 10 years. These ADV-resistant patients had been given only ADV monotherapy (10 mg, oral and once daily), for 2.7 to 4.5 years, with a median duration of 3.4 years. During the course of ADV treatment, the serum creatinine and creatinine clearance rate of all patients were normal.

To explore the association between clinical features and loci mutations, we considered patients' gender, age, end-stage of liver disease, complications of non-alcoholic fatty liver disease (NAFLD), levels of HBV DNA, HBV serum markers, HBV genotypes, and the loci of mutations. Forty-five cases were e-antigen negative and 34 cases were e-antigen positive. There were 16 cases with genotype B and 63 cases with genotype C.

Through inspection of medical records and telephone solicitation, patients were confirmed for inclusion in the study. Each patient provided informed consent before the ADV treatment and preparation of specimens. Based on the results of sequencing drug-resistant HBV mutants, patients were stratified into an rtA181T mutant group and a not-rtA181T mutant group, a single locus mutation group and a multiple loci mutation group, or an rtA181T mutant group and an rtN236T mutant group.

Diagnosis criteria

For all patients, the diagnosis and treatment of their disease complied with the established guidelines for chronic HBV-related diseases[1-5]. The diagnostic criteria for NAFLD met the guidelines for NAFLD[18,19]. End-stage liver diseases included hepatitis cirrhosis, liver failure, and primary hepatocellular carcinoma. Potential patients were excluded if they had undergone combination treatment (that is, combined initially or sometime during the treatment process), or had infections overlapping HBV, or there were other factors leading to abnormal liver function.

Instruments and reagents

Serum HBV DNA was detected by real-time fluorescence-based quantitative PCR[20] (ABI Prism 7500 PCR System, Life Technologies, CA, United States) with a kit provided by Da An Gene of Sun Yat-sen University (approval number: S20040035, 500 copies/mL). HBV serologic markers were analyzed with an Abbott AxSYM System immune assay analyzer (United States). ADV-resistant gene sequencing and genotyping were performed by an ABI 3130 xl sequencing instrument (United States). The fluorescent quantitative nucleic acid amplification and sequencing kit was from Shanghai Shenyou Biological Technology.

Table 1 Loci distribution of adefovir dipivoxil -resistant mutants *n* (%)

ADV-resistant mutants	Number of patients prevalence of patients
rtA181T[1,2] mutant/coexisting mutants[3]	40 (50.63)
rtV214A[1] mutant/coexisting mutants	7 (8.86)
rtN236T[1] mutant/coexisting mutants	5 (6.33)
rtP237H[1] mutant	1 (1.27)
rtL180M[4] + rtM204V[4,5,6] + rtA181T[1,2] coexisting mutants	4 (5.06)
rtL180M[4] + rtT184L[6] coexisting mutants, rtM204V[4,5] + rtV207M/L/I[4] + rtV214A[1] mutant	1 (1.27)
rtL180M[4] + rtM204V[4,5,6] + rtS213T[4] + rtA181T[1,2] mutant	1 (1.27)
rtL180M[4] + rtM204V[4,5,6] + rtS213T[4] + rtQ215S[1] mutant, rtP237H[1] coexisting mutants	1 (1.27)
rtL180M[4] + rtM204V[4,5,6] + rtQ215S[1] + rtT184L[6] mutant	1 (1.27)
rtL180M[4] mutant, rtQ215S[1] coexisting mutants	1 (1.27)
rtA181T[1,2] + rtV214A[1] coexisting mutants	1 (1.27)
rtA181T[1,2] + rtN236T[1] coexisting mutants	2 (2.53)
rtA181T[1] mutant, rtN236T[1] coexisting mutants	1 (1.27)
rtM204V[4,5] + rtA181T[1,2] coexisting mutants	1 (1.27)
rtM204V[4,5] mutant, rtA181T[1,2] coexisting mutants	1 (1.27)
rtM204V[4,5] + rtV207M/L/I[4] + rtQ215S[1] mutant	1 (1.27)
rtM204V[4,5] + rtV214A[1] mutant	1 (1.27)
rtM204V[4,5] mutant, rtA181T[1,2] + rtQ215S[1] coexisting mutants	1 (1.27)
rtM204V[4,5] mutant, rtN238T[1] coexisting mutants	1 (1.27)
rtV207M/L/I[4] + rtA181T[1,2] + rtV214A[1] + rtN236T[1] + rtM250L[6] coexisting mutants	1 (1.27)
rtV207M/L/I[4] + rtA181T[1,2] + rtN236T[1] coexisting mutants	1 (1.27)
rtV207M/L/I[4] + rtV214A[1] coexisting mutants	1 (1.27)
rtV207M/L/I[4] + rtA181T[1,2] + rtN236T[1] coexisting mutants	1 (1.27)
rtS213T[4] + rtA181T[1,2] coexisting mutants	1 (1.27)
rtS213T[4] + rtN236T[1] + rtM250L[6] mutant	1 (1.27)
rtN236T[1] + rtN238T[1] coexisting mutants	1 (1.27)

[1]ADV-associated mutants: rtA181T, rtV214A, rtQ215S, rtN236T, rtP237H, rtN238T; [2]Shared mutants of ADV, LAM, LdT: rtA181T[12]; [3]Coexisting mutants: wild strain and mutant strain coexistence; [4]Lamivudine (LAM)-associated mutants: rtL180M, rtM204V, rtV207M/L/I, rtS213T; [5]Telbivudine (LdT)-associated mutant: rtM204V; [6]Entecavir (ETV)-associated mutants: rtT184L, rtM204V, rtM250L. ADV: Adefovir dipivoxil.

Gene sequencing analysis

To perform quantitative PCR, 50 μL of enriched extraction liquid was added to 50 μL of serum, left for 5 min at 4 ℃ after mixing uniformly, and centrifuged at 20879 × *g* for 10 min. The supernatant was discarded and 40 μL lysing buffer liquid was added and briefly centrifuged. The result was heated for 10 min at 100 ℃, centrifuged at 20879 × *g* for 5 min, and 4 μL to 25 μL added to a reaction mixture. The PCR amplification conditions were: 40 cycles of 94 ℃ for 5 min, 94 ℃ for 7 s, and 60 ℃ for 50 s. For enzymatic hydrolysis of the PCR products, 3 μL of PCR product was mxed with 2 μL of shrimp alkaline phosphatase, kept at 37 ℃ for 60 min and 80 ℃ for 15 min, and stored at 4 ℃. For the sequencing of PCR products, 3 μL of PCR enzymatic products, 1 μL of sequencing reagents (BigDye) and 2 μL of sequencing primers were amplified through 25 cycles of 96 ℃ for 1 min, 96 ℃ for 10 s, 50 ℃ for 5 s, and 60 ℃ for 4 min. Products were purified and stored at 4 ℃ for sequencing.

Liver imaging study

The liver imaging study, which included liver ultrasound or X-ray computed tomography, was performed to assist in the diagnosis of NAFLD[18,19], hepatitis cirrhosis, and primary hepatocellular carcinoma.

Statistical analysis

All data were statistically analyzed using SPSS 18.0 software (SPSS, Chicago, IL, United States). HBV DNA levels are expressed as mean ± SD, determined *via* logarithmic conversion. Continuous normal distribution was analyzed through the independent samples *t*-test, the chi-squared test, or Fisher's exact probability method for rate comparisons. A *P*-value ≤ 0.05 was considered statistically significant.

RESULTS

ADV drug-resistant mutants

The sequencing results revealed that drug-resistant HBV mutants induced by ADV could have either a single locus of mutation or multiple coexisting mutated loci (Table 1). Specifically, the following mutations were found: rtL180M, rtM204V, rtV207M/L/I, rtS213T, rtA181T, rtV214A, rtQ215S, rtN236T, rtP237H, rtN238T, rtT184L, and rtM250L. In these mutations, the drug-resistant HBV mutants related to other NUCs such as lamivudine (LAM) and entecavir (ETV) were also found though patients didn't receive LAM or ETV treatment. The primary mutants in which there was a single mutated locus were rtA181T (40/79, 50.63%), rtV214A (7/79, 8.86%), rtN236T (5/79, 6.33%), and rtP237H (1/79, 1.27%). Twenty-six patients (32.91%) had more than two loci of mutants. The pattern of mutations in ADV-associated mutants were complex and diverse, most patients had coexisting mutations (50, 63.29%).

Table 2 Clinical features of rtA181T mutant and non-mutant rtA181T groups *n* (%)

Clinical features	rtA181T (*n* = 56)	Not rtA181T (*n* = 23)	Statistical value	*P*-value
Gender (M/F)	48/8	18/5	$\chi^2 = 0.659$	0.507
Age (yr, mean ± SD)	43.1 ± 9.3	43.4 ± 11.6	$t = 0.132$	0.896
End-stage liver disease[1]	21 (37.5)	11 (47.83)	$\chi^2 = 0.721$	0.396
NAFLD	21 (37.5)	9 (39.13)	$\chi^2 = 0.018$	0.892
E-antigen (+/-)	27/29	18/5[a]	$\chi^2 = 6.004$	0.023
Genotype (B/C)	7/49	9/14[b]	$\chi^2 = 7.159$	0.007
HBV DNA (log_{10} copy/mL)	5.16 ± 1.23	5.14 ± 1.25	$t = 0.086$	0.932
Duration of treatment (mo, mean ± SD)	41.2 ± 10.1	40.8 ± 9.7	$t = 0.162$	0.872

[1]End-stage liver disease: included hepatitis cirrhosis, liver failure, and primary hepatocellular carcinoma. [a]$P < 0.05$, status of e-antigen in the rtA181T mutant group *vs* in the not-rtA181T mutant group; [b]$P < 0.01$, genotype (B/C) in the rtA181T mutant group *vs* the not-rtA181T mutant group. NAFLD: Non-alcoholic fatty liver disease; HBV: Hepatitis B virus.

Table 3 Clinical features of single mutant and multi-mutant groups *n* (%)

Clinical features	Single mutant (*n* = 53)	Multi-mutants (*n* = 26)	Statistical value	*P*-value
Gender (M/F)	45/8	21/5	$\chi^2 = 0.217$	0.749
Age (yr, mean ± SD)	42.6 ± 10.5	44.5 ± 8.9	$t = 0.806$	0.423
End-stage liver disease[1]	21 (39.62)	11 (42.31)	$\chi^2 = 0.721$	0.396
NAFLD	19 (35.85)	11 (42.31)	$\chi^2 = 0.137$	0.712
E-antigen (+/-)	24/29	12/14	$\chi^2 = 0.005$	1.000
Genotype (B/C)	10/43	6/20	$\chi^2 = 0.022$	0.883
HBV-DNA (log_{10} copy/mL)	5.06 ± 1.24	5.35 ± 1.21	$t = 0.989$	0.326
Duration of treatment (mo, mean ± SD)	38.6 ± 8.2	43.9 ± 10.8[a]	$t = 2.426$	0.018

[1]End-stage liver disease: included hepatitis cirrhosis, liver failure, and primary hepatocellular carcinoma. [a]$P < 0.05$ duration of treatment in the single mutant group *vs* the multi-mutants group. NAFLD: Non-alcoholic fatty liver disease; HBV: Hepatitis B virus.

Comparison of clinical features between the rtA181T and not-rtA181T groups

Based on the results of sequencing, rtA181T was the predominant mutant (56/79, 70.89%). We stratified the patients into rtA181T and not-rtA181T mutation groups, and found no significant differences in regard to gender, age, end-stage liver disease, NAFLD, HBV DNA levels, or duration of treatment with ADV between these two groups (Table 2). However, in patients with the rtA181T mutant, HBV serologic markers were more e-antigen negative and the infective HBV belonged to the C genotype. In patients who did not have the rtA181T mutant, HBV serologic markers were predominately e-antigen positive, and the ratios of infective HBV B genotype and C genotype were higher (χ^2 = 6.004, 7.159; P = 0.023, 0.007).

Comparison of clinical features between the single- and multiple-loci mutation groups

Between the single- and multiple-loci mutation groups, there were no statistically significant differences in patient gender or ages, or rates of end-stage liver disease, or NAFLD (Table 3). There were also no significant differences in HBV serological markers, the genotypes of HBV infection, or HBV DNA levels. However, the duration of treatment with ADV was shorter in the single mutant group than that in the multi-mutant group (t = 2.426, P = 0.018).

Comparison of clinical features between the rtA181T and rtN236T mutant groups

In the current study, there were 50 cases with the rtA181T mutant, 7 cases with the rtN236T mutant, and 6 cases with both the rtA181T and rtN236T mutants (Table 4). Between the first two of these groups there was no significant difference in patients' gender or ages, or rate of end-stage liver disease or NAFLD, or HBV serological markers or DNA levels. However, patients with the rtA181T mutant were infected predominantly by HBV of the C genotype, while patients with rtN236T were infected mainly by HBV of the B genotype (χ^2 = 5.958, P = 0.028).

DISCUSSION

Currently, rtA181V/T/S and rtN236T have been accepted as the primary mutations induced by ADV[1-5]. Other mutations have also been reported, such as I233V[14], rtQ215H/S[15], rtE218G[16], rtL217R, rtV84M, rtS85A, rtV214A, rtP237H, and rtN/H238T/D[21]. In the present study, the ADV-associated mutants rtA181T, rtV214A, rtQ215S, rtN236T, rtP237H, and rtN238T were consistent with literature reports. Although the correlation between other drug-resistant mutants and ADV treatment remains controversial[22], we found high levels of HBV replication in patients who had received ADV. This suggests that viral breakthrough or the development of

Table 4 Clinical features of the rtA181T mutant and rN236T groups *n* (%)

Clinical features	rtA181T mutant (*n* = 50)	rtN236T mutant (*n* = 7)	Statistical value	*P*-value
Gender (M/F)	43/7	7/0	$\chi^2 = 0.069$	0.792
Age (yr, mean ± SD)	42.6 ± 9.4	47.3 ± 12.1	$t = 1.201$	0.235
End-stage liver disease[1]	19 (38)	3 (42.86)	$\chi^2 = 0.026$	1.000
NAFLD	20 (40)	3 (42.86)	$\chi^2 = 0.009$	1.000
E-antigen (+/-)	25/25	2/5	$\chi^2 = 1.131$	0.427
Genotype (B/C)	7/43	5/2[a]	$\chi^2 = 5.958$	0.028
HBV-DNA (log_{10} copy/mL)	5.18 ± 1.23	4.91 ± 1.20	$t = 0.544$	0.588

[1]End-stage liver disease: included hepatitis cirrhosis, liver failure, and primary hepatocellular carcinoma. [a]$P < 0.05$ genotype (B/C) in the rtA181T mutant group *vs* the rtN236T mutant group. NAFLD: Non-alcoholic fatty liver disease; HBV: Hepatitis B virus.

clinical drug resistance and drug-resistant mutants may be related to ADV. We also found that the pattern of mutations in ADV-associated mutants were complex and diverse. Multidrug-resistant mutants (≥ 2 mutated loci) induced by ADV were found in 26 patients (32.91%). Currently, studies on the incidence of ADV-associated multi-drug-resistant mutants have not been reported. We found that rtA181T was the principle mutant in single or multi-ADV-resistant mutations. Interestingly, the number of mutants with rtV214A exceeded those with rtN236T, which is inconsistent with other literature reports[1,2,12]. A study with a large sample size in China is needed to further investigate whether rtA181T + rtV214A mutants correlate with Chinese patients who are mainly infected with genotype C HBV[23-26].

In addition to naturally occurring mutations, HBV produces a variety of mutations to adapt to stresses such as immune pressure from the host or antiviral drugs[27]. In the present study, among 26 patients from whom HBV with multidrug-resistant mutations had been isolated, there were only 5 in which all mutations were related to ADV. Mutations in the isolates from the remaining patients were associated with resistance to LAM, telbivudine (LdT), or ETV, or combinations of these. This implies that HBV may produce mutants that are resistant to other NUCs after treatment with only ADV, because rtA181T/V is a common drug-resistant mutant and may lead to cross-resistance[10].

Studies have found that an rtA181T/V mutation/substitution could generate cross-resistance against tenofovir disoproxil fumarate (TDF), LAM, and LdT[2]. The rtN236T mutation/substitution may have a partial cross-resistance against TDF[28]. Yet rtA181V + rtN236T mutants are highly resistant to LAM, ADV, LdT, and ETV, and with a 10-fold decreased susceptibility to TDF[12]. In addition, rtA181T can cause an sW172* mutation/substitution in the *S* gene, which allows HBV to escape the host's immune response and increases the risk of liver carcinoma[29,30].

HBV genes that encode the polymerase and envelope proteins of HBV overlap, studies have found that an rtA181T mutation could affect HBV replication and protein secretion[31], so there was a higher prevalence of non-rtA181T in e-antigen positive patients. But it needs further basic research into HBV replication and pathogenic mechanisms.

Most patients in this study had coexisting mutations (50, 63.29%). This is an indication of the heterogeneity and complexity of HBV quasispecies, which will obviously influence the antiviral effects of ADV. With more complex kinds of mutations in HBV quasispecies, the use of ADV is more likely to lead to the development of HBV strains with greater cross-drug resistance, thus affecting its antiviral efficacy[32].

In the present study we found no statistically significant differences among the mutations, the mutation frequency with regard to patients' gender or ages, the rates of end-stage of liver diseases or NAFLD, or the levels of HBV DNA. However, the research of others found that chronic HBV male patients with resistant mutations had higher ages and rates of end-stage liver disease and NAFLD than those with no mutations, but the e-antigen positive rate and HBV DNA levels of the former were lower[33]. This suggests that the longer HBV infection persists, the greater the chance that drug resistant mutations will arise as countless passages of HBV interact with the host immune system. Male patients with high numbers of ADV-resistant mutations may correlate with high rates of incidence and chronicity of HBV infection, and high frequency of HBV activity[34].

A consensus has not been reached regarding the association between HBV genotype and ADV resistance. Some studies suggest that there is no correlation[35,36], while others suggest that patients with HBV genotype D are prone to develop ADV resistance[37]. In China, most HBV genotypes are B or C[24-26]. Other relevant studies have found that in Chinese patients, the ratio of genotype C HBV was relatively higher in those with ADV-associated mutations than in those without ADV-associated mutations[33]. This indicates that patients with genotype C HBV infection readily have ADV-resistant mutants. This may be because the majority of HBV patients in China are infected with genotype C HBV[24-26], genotype C HBV readily generates drug-resistant strains[26], or patients infected with genotype C HBV are more prone to advanced liver disease[38]. Patients with the HBV mutant rtA181T are predominantly infected by e-antigen negative and genotype C HBV, while patients with rtN236T are predominantly infected by genotype B HBV. This is consistent with the increased risk of liver carcinoma in

those with the rtA181T HBV mutant[29,30], and the association of the genotype C HBV with more advanced liver disease[38]. This suggests that for patients with chronic HBV infection with both rtA181T and genotype C, disease progression may be exacerbated. Thus, ADV monotherapy should not be used for patients with chronic genotype C HBV infection.

Overall, patients with chronic HBV infection may have ADV- or other NUC-resistant HBV mutants prior to antiviral therapy. Mutations in ADV-resistant HBV strains are complex and diverse, and always accompanied by other NUC-resistant mutants. In China, ADV is used widely and the public health problems caused by ADV resistance and cross resistance are becoming more severe. Therefore, testing for resistant mutants is recommended prior to antiviral treatment. First-line antiviral treatment and customized medication based on the clinical characteristics of patients is important to avoid subsequent improper drug use and the need for salvage treatment. At the same time, management of antiviral therapy should be improved by encouraging medication compliance and avoiding short-term withdrawal.

ACKNOWLEDGMENTS

We thank Medjaden Bioscience Limited for assisting in the preparation of this manuscript.

COMMENTS

Background

The treatment of liver diseases associated with chronic hepatitis B virus (HBV) infection has become a major clinical challenge, partly due to the many drug-resistant HBV mutants induced by wide application of nucleoside/nucleotide analogues (NUCs). In China, adefovir dipivoxil (ADV) is routinely applied in initial monotherapy for HBV-related liver disease, as well as monotherapy subsequent to treatment with other NUCs for which the virus developed resistance. As a result, HBV resistance to ADV has become more prominent.

Research frontiers

Currently, rtA181V/T/S and rtN236T have been accepted as the primary drug-resistant HBV mutations induced by ADV, and other mutations have also been reported. An rtA181T/V mutation/substitution could generate cross-resistance against tenofovir disoproxil fumarate, lamivudine (LAM), and telbivudine (LdT). The rtN236T mutation/substitution may have a partial cross-resistance against TDF. Yet rtA181V + rtN236T mutants are highly resistant to LAM, ADV, LdT, and entecavir, and with a 10-fold decreased susceptibility to TDF. In addition, rtA181T can cause an sW172* mutation/substitution in the *S* gene, which allows HBV to escape the host's immune response and increases the risk of liver carcinoma. In this study, the authors demonstrate that ADV-resistant patients were found either with a single mutated locus, or with coexisting mutated loci, and drug-resistant HBV mutants are complex and diverse.

Innovations and breakthroughs

Most current studies focus on ADV drug resistance and optimal treatment. This is the first study to report that ADV-resistant HBV mutants are complex and diverse, either with a single mutated locus, or with coexisting mutated loci. Patients with the rtA181T mutant were primarily infected by genotype C and e-antigen negative HBV, while patients with the rtN236T mutant were primarily infected by genotype B HBV. The duration of treatment with ADV was shorter in the single mutant group than that in the multi-mutant group.

Applications

The investigation of exhaustive resistant mutants induced by ADV will help to optimize the management of ADV-treated patients. In China, ADV is used widely and the public health problems caused by ADV resistance and cross resistance are becoming more severe. Therefore, testing for resistant mutants is recommended prior to antiviral treatment. Personalizing medication based on the clinical characteristics of patients is important to avoid subsequent improper drug use and the need for salvage treatment. At the same time, management of antiviral therapy should be improved by encouraging medication compliance and avoiding short-term withdrawal.

Terminology

Coexisting mutants and cross resistance are different manifestations of drug resistance. Coexisting mutants refers to wild strain and mutant strain coexistence, and cross resistance refers to mutation selected by one antiviral agent that also confers resistance to other antiviral agents. End-stage liver disease includes hepatitis cirrhosis, liver failure, and primary hepatocellular carcinoma.

Peer review

The authors investigated the exhaustive resistant mutants induced by ADV and the association between the clinical features of patients and these drug-resistant HBV mutants induced by ADV. The analysis revealed that ADV-resistant HBV mutants are complex and diverse, either with a single mutated locus, or with coexisting mutated loci. Patients with the rtA181T mutant were primarily infected by genotype C and e-antigen negative HBV, while patients with the rtN236T mutant were primarily infected by genotype B HBV. The results are interesting and may help to optimize the management of ADV-treated patients.

REFERENCES

1 **European Association For The Study Of The Liver**. EASL clinical practice guidelines: Management of chronic hepatitis B virus infection. *J Hepatol* 2012; **57**: 167-185 [PMID: 22436845 DOI: 10.1016/j.jhep.2012.02.010]
2 **Liaw YF**, Kao JH, Piratvisuth T, Chan HL, Chien RN, Liu CJ, Gane E, Locarnini S, Lim SG, Han KH, Amarapurkar D, Cooksley G, Jafri W, Mohamed R, Hou JL, Chuang WL, Laurentius A, Lesmana, Jose D, Sollano, Suh DJ, Omata M. Asian-Pacific consensus statement on the management of chronic hepatitis B: a 2012 update. *Hepatol Int* 2012; **6**: 531-561 [DOI: 10.1007/s12072-012-9365-4]
3 **Keeffe EB**, Dieterich DT, Han SH, Jacobson IM, Martin P, Schiff ER, Tobias H. A treatment algorithm for the management of chronic hepatitis B virus infection in the United States: 2008 update. *Clin Gastroenterol Hepatol* 2008; **6**: 1315-1341; quiz 1286 [PMID: 18845489 DOI: 10.1016/j.cgh.2008.08.021]
4 **Lok AS**, McMahon BJ. Chronic hepatitis B: update 2009. *Hepatology* 2009; **50**: 661-662 [PMID: 19714720 DOI: 10.1002/hep.23190]
5 **Chinese Society of Hepatology and Chinese Society of Infectious Diseases, Chinese Medical Association**. [The guideline of prevention and treatment for chronic hepatitis B (2010 version)]. *Zhonghua Liuxing Bingxue Zazhi* 2011; **32**: 405-415 [PMID: 21569677]
6 **Wong VW**, Wong GL, Tse CH, Yuen LK, Chan HY, Locarnini SA, Chan HL. Antiviral drug resistance testing in patients with chronic hepatitis B. *Dig Dis Sci* 2012; **57**: 221-231 [PMID: 21743991 DOI: 10.1007/s10620-011-1816-6]
7 **Lok AS**, Zoulim F, Locarnini S, Bartholomeusz A, Ghany MG, Pawlotsky JM, Liaw YF, Mizokami M, Kuiken C. Antiviral drug-resistant HBV: standardization of nomenclature and assays and recommendations for management. *Hepatology* 2007; **46**: 254-265 [PMID: 17596850 DOI: 10.1002/hep.21698]
8 **Locarnini S**, Hatzakis A, Heathcote J, Keeffe EB, Liang TJ, Mutimer D, Pawlotsky JM, Zoulim F. Management of antiviral resistance in patients with chronic hepatitis B. *Antivir Ther* 2004; **9**: 679-693 [PMID: 15535405]
9 **Locarnini S**, Warner N. Major causes of antiviral drug resistance and implications for treatment of hepatitis B virus monoinfection and coinfection with HIV. *Antivir Ther* 2007; **12** Suppl 3: H15-H23 [PMID: 18284179]
10 **Locarnini S**. Primary resistance, multidrug resistance, and cross-resistance pathways in HBV as a consequence of treatment failure. *Hepatol Int* 2008; **2**: 147-151 [PMID: 19669299 DOI: 10.1007/s12072-008-9048-3]
11 **Wang XY**, Wei ZM, Wu GY, Wang JH, Zhang YJ, Li J, Zhang

HH, Xie XW, Wang X, Wang ZH, Wei L, Wang Y, Chen HS. In vitro inhibition of HBV replication by a novel compound, GLS4, and its efficacy against adefovir-dipivoxil-resistant HBV mutations. *Antivir Ther* 2012; **17**: 793-803 [PMID: 22668794 DOI: 10.3851/IMP2152]

12 **Qi X**, Xiong S, Yang H, Miller M, Delaney WE. In vitro susceptibility of adefovir-associated hepatitis B virus polymerase mutations to other antiviral agents. *Antivir Ther* 2007; **12**: 355-362 [PMID: 17591025]

13 **Zhao P**, Wang C, Huang L, Xu D, Li T. Comparison of rescue strategies in lamivudine-resistant patients with chronic hepatitis B. *Antiviral Res* 2012; **96**: 100-104 [PMID: 22960601 DOI: 10.1016/j.antiviral.2012.08.008]

14 **Curtis M**, Zhu Y, Borroto-Esoda K. Hepatitis B virus containing the I233V mutation in the polymerase reverse-transcriptase domain remains sensitive to inhibition by adefovir. *J Infect Dis* 2007; **196**: 1483-1486 [PMID: 18008227]

15 **Micco L**, Fiorino S, Loggi E, Lorenzini S, Vitale G, Cursaro C, Riili A, Bernardi M, Andreone P. Polymorphism rtQ215H in primary resistance to adefovir dipivoxil in hepatitis B virus infection: a case report. *BMJ Case Rep* 2009; **2009**: bcr06.2008.0287 [PMID: 21686872 DOI: 10.1136/bcr.06.2008.0287]

16 **Liu LJ**, Wang JH, Du SC, Tian JH, Yang RF, Wei L. rtE218G, a novel hepatitis B virus mutation with resistance to adefovir dipivoxil in patients with chronic hepatitis B. *J Viral Hepat* 2010; **17** Suppl 1: 66-72 [PMID: 20586936 DOI: 10.1111/j.1365-2893.2010.01273.x]

17 **Miyakawa Y**, Mizokami M. Classifying hepatitis B virus genotypes. *Intervirology* 2003; **46**: 329-338 [PMID: 14688448 DOI: 10.1159/000074988]

18 **Chalasani N**, Younossi Z, Lavine JE, Diehl AM, Brunt EM, Cusi K, Charlton M, Sanyal AJ; American Gatroenterological Association; American Association for the Study of Liver Disease; American College of Gatroenterology. The diagnosis and management of non-alcoholic fatty liver disease: practice guideline by the American Gastroenterological Association, American Association for the Study of Liver Diseases, and American College of Gastroenterology. *Gastroenterology* 2012; **142**: 1592-1609 [PMID: 22656328 DOI: 10.1053/j.gastro.2012.04.001]

19 **Fan JG**, Jia JD, Li YM, Wang BY, Lu LG, Shi JP, Chan LY; Chinese Association for the Study of Liver Disease. Guidelines for the diagnosis and management of nonalcoholic fatty liver disease: update 2010: (published in Chinese on Chinese Journal of Hepatology 2010; 18: 163-166). *J Dig Dis* 2011; **12**: 38-44 [PMID: 21276207 DOI: 10.1111/j.1751-2980.2010.00476.x]

20 **Jiang SW**, Hu AR, Zhou WH, Ying H, Ding SX, Weng PJ. [Comparison of two methods for the detection of hepatitis B virus (HBV) DNA in serum samples]. *Zhonghua Ganzangbing Zazhi* 2009; **17**: 952-953 [PMID: 20038344]

21 **Locarnini S**, Mason WS. Cellular and virological mechanisms of HBV drug resistance. *J Hepatol* 2006; **44**: 422-431 [PMID: 16364492]

22 **Borroto-Esoda K**, Miller MD, Arterburn S. Pooled analysis of amino acid changes in the HBV polymerase in patients from four major adefovir dipivoxil clinical trials. *J Hepatol* 2007; **47**: 492-498 [PMID: 17692425]

23 **Locarnini SA**, Qi X, Arterburn S, Snow A, Brosgart CL, Currie G, Wulfsohn M, Miller MD, Xiong S. Incidence and predictors of emergence of adefovir resistant HBV during four years of adefovir dipivoxil (ADV) therapy for patients with chronic hepatitis B (CHB). *J Hepatol* 2005; **42**: 17

24 **Hu Y**, Zhang WL, Xie SL, Zhao Y, Hu JL, Cai XF, Lai GQ, Huang AL. An improved reverse dot hybridization for simple and rapid detection of adefovir dipivoxil-resistant hepatitis B virus. *Genet Mol Res* 2012; **11**: 53-60 [PMID: 22290465 DOI: 10.4238/2012.January.9.6]

25 **Moskovitz DN**, Osiowy C, Giles E, Tomlinson G, Heathcote EJ. Response to long-term lamivudine treatment (up to 5 years) in patients with severe chronic hepatitis B, role of genotype and drug resistance. *J Viral Hepat* 2005; **12**: 398-404 [PMID: 15985011]

26 **Fung SK**, Chae HB, Fontana RJ, Conjeevaram H, Marrero J, Oberhelman K, Hussain M, Lok AS. Virologic response and resistance to adefovir in patients with chronic hepatitis B. *J Hepatol* 2006; **44**: 283-290 [PMID: 16338024]

27 **Ji F**, Zhou L, Ma S, Li F, Ding H, Zeng C. Dynamic changes of HBV quasispecies and deletion patterns in a chronic hepatitis B patient. *J Med Virol* 2009; **81**: 1551-1559 [PMID: 19626620 DOI: 10.1002/jmv.21595]

28 **Zoulim F**, Locarnini S. Management of treatment failure in chronic hepatitis B. *J Hepatol* 2012; **56** Suppl 1: S112-S122 [PMID: 22300461 DOI: 10.1016/S0168-8278(12)60012-9]

29 **Warner N**, Locarnini S. The antiviral drug selected hepatitis B virus rtA181T/sW172* mutant has a dominant negative secretion defect and alters the typical profile of viral rebound. *Hepatology* 2008; **48**: 88-98 [PMID: 18537180 DOI: 10.1002/hep.22295]

30 **Yeh CT**, Chen T, Hsu CW, Chen YC, Lai MW, Liang KH, Chen TC. Emergence of the rtA181T/sW172* mutant increased the risk of hepatoma occurrence in patients with lamivudine-resistant chronic hepatitis B. *BMC Cancer* 2011; **11**: 398 [PMID: 21933446 DOI: 10.1186/1471-2407-11-398]

31 **Zoulim F**, Locarnini S. Hepatitis B virus resistance to nucleos(t)ide analogues. *Gastroenterology* 2009; **137**: 1593-1608.e1-2 [PMID: 19737565 DOI: 10.1053/j.gastro.2009.08.063]

32 **Nishijima N**, Marusawa H, Ueda Y, Takahashi K, Nasu A, Osaki Y, Kou T, Yazumi S, Fujiwara T, Tsuchiya S, Shimizu K, Uemoto S, Chiba T. Dynamics of hepatitis B virus quasispecies in association with nucleos(t)ide analogue treatment determined by ultra-deep sequencing. *PLoS One* 2012; **7**: e35052 [PMID: 22523569 DOI: 10.1371/journal.pone.0035052]

33 **Hu AR**, Jiang SW, Li L, Ding SX, Hu YR, Liang XY. [Clinical analysis of hepatitis B virus mutations related to adefovir dipivoxil among patients with chronic hepatitis B virus infection in eastern Zhejiang province]. *Zhonghua Yixue Zazhi* 2012; **92**: 1878-1881 [PMID: 23134956]

34 **Song GJ**, Feng B, Rao HY, Wei L. Etiological features of cirrhosis inpatients in Beijing, China. *Chin Med J* (Engl) 2013; **126**: 2430-2434 [PMID: 23823813 DOI: 10.3760/cma.j.issn.0366-6999.20130184]

35 **Zeng G**, Wang Z, Wen S, Jiang J, Wang L, Cheng J, Tan D, Xiao F, Ma S, Li W, Luo K, Naoumov NV, Hou J. Geographic distribution, virologic and clinical characteristics of hepatitis B virus genotypes in China. *J Viral Hepat* 2005; **12**: 609-617 [PMID: 16255762 DOI: 10.1111/j.1365-2893.2005.00657.x]

36 **Yang JX**, Liu BM, Li XG, Yan CH, Xu J, Sun XW, Wang YH, Jiao XJ, Yan L, Dong JP, Hou CS, Abuduheilili X, Li T, Zhuang H. Profile of HBV antiviral resistance mutations with distinct evolutionary pathways against nucleoside/nucleotide analogue treatment among Chinese chronic hepatitis B patients. *Antivir Ther* 2010; **15**: 1171-1178 [PMID: 21149924 DOI: 10.3851/IMP1677]

37 **Sun J**, Hou JL. Management of chronic hepatitis B: experience from China. *J Viral Hepat* 2010; **17** Suppl 1: 10-17 [PMID: 20586929 DOI: 10.1111/j.1365-2893.2010.01274.x]

38 **Wong GL**, Chan HL, Yiu KK, Lai JW, Chan VK, Cheung KK, Wong EW, Wong VW. Meta-analysis: The association of hepatitis B virus genotypes and hepatocellular carcinoma. *Aliment Pharmacol Ther* 2013; **37**: 517-526 [PMID: 23305043 DOI: 10.1111/apt.12207]

P- Reviewer: Kanda T, Tai DI **S- Editor**: Ma YJ **L- Editor**: A **E- Editor**: Wang CH

Published by **Baishideng Publishing Group Inc**
8226 Regency Drive, Pleasanton, CA 94588, USA
Telephone: +1-925-223-8242
Fax: +1-925-223-8243
E-mail: bpgoffice@wjgnet.com
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
http://www.wjgnet.com

ISSN 1007-9327