Original Article

Detection of Mutations Resistant to Lamivudine or Adefovir in HBV and Its Management

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Objective Nucleos(t)ide analogues (NAs) naïve chronic hepatitis B(CHB) patients were given rescue combination therapy after drug resistance to lamivudine or adefovir. Evolution of HBV mutation patterns and its impact on antiviral effects were studied.

Methods Total of 142 naïve CHB patients treated with lamivudine were randomly divided into two groups when lamivudine resistance occurred. One group was added with adefovir, the other was switched to entecavir and adefovir. Seventy-two naïve CHB patients treated with adefovir were randomly divided into two groups when adefovir resistance occurred. One group was added with lamivudine, the other was added with entecavir. HBV polymerase reverse transcriptase mutations associated with resistance were analysed before and after 48 weeks of rescue therapy, respectively.

Results The mutation patterns of M204V/I, M204V+L180M were predominantly found in CHB patients after lamivudine resistance. Meanwhile, the entecavir resistance mutation patterns were also detected. Therefore, patients with lamivudine resistance could develop more diverse drug resistance mutations if they were switched to entecavir and adefovir. The mutation patterns of rtA181 were predominantly found in CHB patients after adefovir resistance and rescue therapy with add-on entecavir was more effective than with add-on lamivudine.

Conclusions Resistance mutation analysis should help to choose NAs, reduce resistance and enhance antiviral effects.

Key words: Lamivudine; Adefovir; Resistance; Rescue therapy

A major concern with long-term NAs treatment is the selection of antiviral-resistant mutations. Chronic hepatitis B (CHB) patients with confirmed NAs - resistance should receive rescue therapy with other antiviral agent that is not cross-resistant. Total of 142 naïve CHB patients treated with lamivudine and 72 naïve CHB patients treated with adefovir were enrolled in our study when lamivudine resistance and adefovir resistance occurred, respectively. Evolution of HBV mutation patterns before and after the rescue therapy as well as its impact on antiviral effects were analyzed.

PATIENTS AND METHODS

Total of 142 naïve CHB patients (males 98, females 44, average age 36.5 ± 10.3 years) with lamivudine resistance during lamivudine treatment and 72 naïve CHB patients (male 50, female 22, average age

43.1 ± 15.7 years) with adefovir resistance during adefovir treatment in our hospital from September 2009 to December 2012 were included in our study. Those 142 naïve CHB patients with lamivudine resistance were randomly divided into two groups. One group was added with adefovir as rescue therapy, the other was switched to entecavir and adefovir. 72 naïve CHB patients with adefovir resistance during adefovir treatment were randomly divided into two groups. One group was added with lamivudine, the other was added with entecavir. HBV polymerase reverse transcriptase mutations associated with resistance were analysed before and after 48 weeks of rescue therapy, respectively.

The HBV DNA levels were measured by FQ-PCR using PE-5700 machine of ABI and PCR kit produced by Shenzhen Piji Company with a low limit of quantification of 500 copies/ml and a upper limit of quantification of 5×10^7 copies/ml. A nested PCR technique was used to amplify specific HBV gene fragments with HBV DNA extraction kit from Shanghai Fosun Company and S1000 PCR Thermal

Cycler from Bio-Rad. Pyrosequencing was used to detect HBV polymerase reverse transcriptase mutations associated with drug resistance by PyroMark Q96 ID instrument and PyroMark Gold Q96 Reagents (QIAGEN). A total of 11 mutations were tested: rtL180M, rtM204V/I, rtV173L, rtI169T, rtS202G (V/C/I), rtM250V (L/I), rtA181V/T, rtN236T, rtT184A (G/I/S/L/F), rtA194T/M and rtI233V.

Virologic response is defined as the absence of HBV DNA detectable by real-time PCR or a decrease in serum HBV DNA by $\geq 2 \log_{10}$ copies/ml. Virologic nonresponse is defined as the inability of NAs to reduce serum HBV DNA by $2 \log_{10}$ copies/ml after the first 24 weeks of treatment with good compliance. Virologic breakthrough is defined as an increase in serum HBV DNA by $\geq 1 \log_{10}$ copies/ml from nadir in two consecutive samples 1 month apart in patients who have responded and have been compliant with antiviral medication(s).

All analyses were conducted using SPSS version 13.0. Two independent samples *t* test was used for mean differences between groups. χ^2 test and Fisher exact test were used for continuous variables.

RESULTS

It was showed in Table 1 the lamivudine resistance

Table 1. Mutation patterns after lamivudine resistance

Group	Mutation patterns	Cases	Percentages (%)
Lamivudine resistance	M204V	32	22.5
	M204I	18	12.7
	M204V+L180 M	50	35.2
	M204V+L180 M+V173L	12	8.5
	L180 M	2	1.4
	M204V+L180 M+S202G	8	5.6
	M204V+T184G+L180M	8	5.6
	M204V+V173L	12	8.5

Table 2. Baseline characteristics of 142 CHB patients with lamivudine resistance

	Cases	Age (years)	ALT (U/L)	HBV-DNA ($\log_{10}$ copies/ml)
Lamivudine and adefovir	72	33.1 $\pm$ 12.7 (19 - 55)	67.1 $\pm$ 40.5 (18 - 204)	5.36 $\pm$ 0.89
Entecavir and adefovir	70	33.5 $\pm$ 9.8 (21 - 68)	73.8 $\pm$ 57.0 (21 - 443)	5.29 $\pm$ 1.01
<i>P</i> value		<i>t</i> = 0.836 <i>P</i> = 0.05	<i>t</i> = 1.058 <i>P</i> = 0.05	<i>t</i> = 0.412 <i>P</i> = 0.05

mutation patterns that were detected in 142 naïve CHB patients during lamivudine treatment. M204V/I was found in 98.6% (140/142) of patients, L180M 56.3% (80/142), V173L 16.9% (24/142), A181V 2.8% (4/142), N236T 2.8% (4/142), T184G 5.6% (8/142), S202G 5.6% (8/142). M204V/I and M204V+L180 were predominant. Meanwhile, entecavir associated resistance mutations, M204V+L180 M+S202G (8/142) and M204V+T184G+L180 M (8/142), were also found in our study.

Total of 142 naïve CHB patients treated with lamivudine were randomly divided into two groups when lamivudine resistance occurred. One group were added with adefovir, the other were switched to entecavir and adefovir. It was showed in Table 2 the baseline characteristics of the 142 patients.

After 48 months of rescue therapy, undetectable serum HBV DNA was noted in 62 (86.1%) patients in lamivudine and adefovir treatment group, while 68 (97.1%) patients in entecavir and adefovir treatment group. However, the difference between the two groups was not significant ($\chi^2 = 2.099$, *P* > 0.05). Serum HBV DNA became undetectable in patients with lamivudine resistance mutation patterns, M204V, M204I, M204V+L180M+V173L, L180M, M204V+V173L after 48 weeks of rescue therapy. In lamivudine and adefovir treatment group, serum HBV DNA was detectable in 3 patients with mutation pattern M204V+L180M at week 48 and mutation pattern in one patient changed to A181T+M204V+L180 M+V173L while the other 2 unchanged. 2 patients with mutation

Table 3. Mutation patterns after adefovir resistance

Group	Mutation patterns	Cases	Percentages (%)
Adefovir resistance	A181V+N236T	12	16.7
	A181T+N236T	6	8.3
	N236T	8	11.1
	A181V	34	47.2
	N236T+L180 M	12	16.7

Table 4. Baseline characteristics of 72 CHB patients with adefovir resistance

	Cases	Age (years)	ALT (U/L)	HBV-DNA ($\log_{10}$ copies/ml)
Adefovir and lamivudine	36	42.5 $\pm$ 18.3 (29-67)	45.8 $\pm$ 28.0 (16-119)	4.31 $\pm$ 0.95
Adefovir and entecavir	36	39.7 $\pm$ 20.1 (22-65)	50.5 $\pm$ 31.7 (19-220)	4.33 $\pm$ 0.76
<i>P</i> value		<i>t</i> = 1.966 <i>P</i> > 0.05	<i>t</i> = 0.703 <i>P</i> > 0.05	<i>t</i> = 1.075 <i>P</i> > 0.05

patten M204V+L180 M+S202G and 5 patients with M204V+T184G+L180M were found with detectable serum HBV-DNA and unchanged mutation patters. Serum HBV DNA was detectable in 2 patients with mutation patten M204V+T184G+L180M in entecavir and adefovir treatment group, and mutation patten in one patient changed to M204V+L180M+T184G/I+A181T.

It was showed in Table 3 that the adefovir resistance mutation patters that were detected in 72 naïve CHB patients during adefovir treatment. A181V was found in 63.9% (46/72) of patients, N236T 52.8% (38/72), A181T 8.3% (6/72), L180 M 16.7 (12/72).

72 naïve CHB patients treated with adefovir were randomly divided into two groups when adefovir resistance occurred. One group was added with lamivudine, the other with entecavir. Table 4 showed the baseline characteristics of the 72 patients.

After 48 months of rescue therapy, undetectable serum HBV DNA was noted in 19 patients in adefovir and lamivudine treatment group, while 28 patients in adefovir and entecavir treatment group. The difference between the two groups was significant ($\chi^2 = 4.963$, $P < 0.05$). In adefovir and lamivudine treatment group, patients with mutation patten A181V+N236T or A181T+N236T were found detectable serum HBV DNA and with unchanged mutation patters at week 48. Among patients with mutation patten A181V, 9 were negative in serum HBV DNA and one was found with mutation patten A181V+M204V+L180 M. 6 patients with mutation patten A181V+N236T and 2 patients with mutation patten A181T+N236T in adefovir and entecavir treatment group were found detectable serum HBV DNA and with unchanged mutation patters at week 48.

DISCUSSION

The primary lamivudine resistance mutation is rtM204V/I/S accompanied by compensatory mutations rtL180M or V173L and so on. Other nucleos(t)ide analogues resistant mutants have been reported to be selected after emergence of lamivudine resistance.^{1,2} Our study showed that mutations resistant to both lamivudine and entecavir existed in lamivudine resistant CHB patients before rescue therapy and its mutation patten was M204V+L180M+S202G, M204V+T184G+L180M. HBV DNA negativity rate was insignificantly different between lamivudine and adefovir treatment group and entecavir and adefovir treatment group after 48 weeks

of rescue therapy. But notably, mutations resistant to both lamivudine and adefovir developed in patients with poor response in lamivudine and adefovir treatment group. Multidrug resistance for lamivudine, adefovir and entecavir happened in patients with poor response in entecavir and adefovir treatment group.

The primary adefovir resistance mutation is rtA181V/T and rtN236T. RtA181V/T mutant has reduced susceptibility to lamivudine and adefovir. But the mutant with single rtN236T is susceptible to lamivudine but has a significant decrease in susceptibility to adefovir.³ According to EASL 2009 CHB guidelines, rtA181 mutant is susceptible to telbivudine and entecavir.⁴ It is also confirmed by a study from China that to add entecavir or switch to entecavir is more effective than to add telbivudine.⁵ Our study found rescue therapy with add-on entecavir was more effective than with add-on lamivudine for CHB patients with adefovir resistance and multidrug resistance mutations were detected in patients with poor response.

Nowadays naïve CHB patients in China are predominantly treated with antiviral therapy with low potency and high rate of genotypic resistance. Multidrug resistance could develop when various NAs have been experienced during combination therapy or sequential treatment because of poor response, resistance mutations or other reasons. Avoidance of multidrug resistance is very important. So we suggest that all HBV polymerase reverse transcriptase mutations associated with resistance should be completely analysed instead of solely detection of resistant mutations associated with experienced NAs. Resistance mutation analysis should help to choose NAs, reduce resistance and enhance antiviral effects. When rtM204 mutation happens during lamivudine treatment, combination therapy with adefovir is definitively effective, while rescue therapy with a switch to entecavir and adefovir fails to increase effect but produces more diverse drug resistance mutations. When rtA181 mutation happens during adefovir treatment, combination therapy with entecavir is more effective than with lamivudine. Multidrug resistance mutations were not found in CHB patients of our study treated with rescue therapy with add-on entecavir after adefovir resistance. Whether a follow-up time of 48 weeks is not long enough for multidrug resistance to be detected is unknown and more research is needed in order to understand it.

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